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Journal of DENTAL RESEARCH

Journal of DENTAL RESEARCH

FOUNDED BY WILLIAM J. GIES IN 1919

Published by the International Association for Dental Research

This *Journal* is dedicated to the dissemination of knowledge pertaining to the oral cavity, its contents and contiguous parts, and its relation to and effect on the total human organism. The *Journal* publishes original investigations in these and related fields. Articles are accepted only if they are contributed solely to this *Journal*.

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Editorial Views and News

In this July-August issue of the *Journal,* there appears the complete proceedings of the Thirty-eighth Meeting of the International Association for Dental Research. As all who attended will recall, this was held March 17–20 of this year in Chicago. Moreover, the meeting proved to be the fourteenth time the site has been the city of Chicago. Since the founding of the I.A.D.R. in 1920, Chicago has been host to the dental research group in 1924, 1925, 1926, 1929, 1933 (nominal meeting only), 1934, 1935, 1943, 1944, 1945 (curtailed meeting of only one day—*en guerre*), 1947, 1949, 1955, and 1960.

In the pages that follow there can be observed the very noteworthy presidential address of the current I.A.D.R. President, Holmes T. Knighton. Clinical research in dentistry is not only a difficult area of human endeavor, but it is even a difficult topic upon which to build an expository address or essay. Holmes Knighton, well known for his research in the clinical field, now proves his ability to present the case for more and better research in the clinical area. Beyond the presidential address the next ten pages are devoted to the Proceedings as compiled by the Secretary-Treasurer, Dan Y. Burrill. The considerable detail of all the Council actions and Executive Sessions are all duly published for the record as well as for perusal of members and other readers. It is very apparent to the Editor that, if all I.A.D.R. Councillors and other members would read and assimilate these valuable Council records of discussions and actions, much time could be saved and wiser decisions made at subsequent Council meetings.

This year, more so than in other years, the abstracts of papers presented at the annual meeting seem to occupy a great deal of space in the *Journal* (117 pages plus the Index). Furthermore, there were a total of 319 abstracts. While their publication leaves less room for manuscripts in this fourth number of the *Journal,* these abstracts sometimes are the only record of an individual's research work. In too many instances a final paper is never consummated on the part of the author or is never published because of the inferior nature of the manuscript.

For researchers not fully acquainted with manuscript preparation (and it is somewhat of an art, though readily acquirable even by the most introvertive of scientists), the Editor would refer potential authors to the pages appearing in the front section of the January-February, 1960, issue of the *Journal,* entitled "Instructions to Authors." Here, as well as in the Editor's Report, appearing in the Proceedings of this issue, the general effort is made to persuade authors and potential authors to conform to certain aspects and standards of manuscript preparation. Full co-operation would greatly facilitate the sometimes laborious work of the editorial staff in synthesizing a journal out of a conglomeration of unconforming papers.

Bibliography on Saliva.—While many other journals dealing with oral phenomena have the space and general policy to carry announcements, book reviews, and other more or less interesting features for their readers, the *Journal of Dental Research* has always tried to devote as many pages as possible to the straight and, we believe, significant role of publishing worthwhile research papers in the oral field.

Nevertheless, when a published compilation appears, such as the *Bibliography on Saliva* currently available to research workers, mention of this fact must be made. This very comprehensive volume was compiled under contract with the Office of Naval Research by the Bibliography Section, Science and Technology Division of the Library of Congress. It was published in March, 1960, by the O.N.R., Department of the Navy, Washington, D.C., and is available for the nominal sum of $3.00 at the U.S. Government Printing Office. This publication should prove to be a very useful reference work, although the inclusion of an author cross-index would have made it even more valuable. The abstracting of articles was begun in 1950 and covers the literature through 1957, with additional coverage of only such specific publications as the *Journal of Dental Research* and *Oral Surgery, Oral Medicine, and Oral Pathology* through 1958. This unique "compilation is not intended as a textbook on saliva, but is a guide to the existing literature in this extremely important and growing area of biologic research."

—F. J. O.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Thirty-ninth Annual Meeting, March 23, 24, 25, and 26, 1961
Hotel Statler, Boston, Massachusetts

Abstracts of papers for presentation at the thirty-ninth annual meeting of the I.A.D.R. in Boston, Massachusetts, March 23, 24, 25, and 26, 1961, must be submitted on *special forms provided by the Association.*

Abstracts must fulfil the requirements stated on the form and must be fully ready, in form and substance, for inclusion in the preprinted abstracts. The preprinted abstracts will be prepared photographically from the abstracts as submitted—no editing or corrections will be possible. Abstracts not in proper form will be returned to the authors if time allows.

The abstracts of papers presented at the meeting will be formally published in the *Journal of Dental Research* after the meeting.

The deadline for receipt of abstracts will be *December 1, 1960.* Mail all abstracts to: James A. English, Capt. DC USN, Chairman of Program Committee, Office of Naval Research, Building T-3, Navy Department, Washington 25, D.C.

DAN Y. BURRILL
Secretary-Treasurer

*Presidential Inaugural Address**

An Evaluation of Clinical Research in Dentistry

HOLMES T. KNIGHTON
Medical College of Virginia, Richmond, Virginia

There has been a significant growth in the field of dental research during the last thirty years and particularly since 1950. It is noted in Kesel's[1] 1959 report that in 1936 only 12 of 43 American dental schools had funds for research. The total of research budgets for these schools was $41,370. In sharp contrast, as of June, 1959, the National Institute of Dental Research's budget for research grants to different institutions was $6,226,000. This, of course, is in addition to dental research funds from state budgets, commercial companies, and philanthropic groups. Furthermore, as of November, 1959, 357 N.I.D.R. research grants were distributed to 91 institutions in the United States, including 43 dental schools. All this is adequate proof that funds are now available and well distributed in an attempt to encourage continuing research.

Evidence that money available for dental research is being used wisely is suggested by the following: an increased membership in the International Association of Dental Research from 53 in 1920 to well over 1,000 in 1960, presentation of from 250 to 300 research papers at recent I.A.D.R. meetings, and numerous reports by dental researchers published in various scientific journals.

In addition to continued financial aid, what are our needs for further improving the status of dental research? Obviously, many points could be discussed in answering this question; however, I shall confine my remarks largely to the field of clinical research. For convenience of discussion, research will be divided into the broad divisions of basic and applied research. Basic research will be considered as that concerned chiefly with such sciences as chemistry, physics, and various fields of biology. Such basic research is often performed with an idealistic goal in mind and with little thought relative to so-called practical applications. In contrast, the chief aim of applied research is slanted toward an immediate practical application. Clinical research is an example of applied research.

Up until relatively recent years, dentistry had not contributed much to basic research. However, based on numerous recent publications, it would seem that dental researchers are now making significant strides in this area. There is still room for much progress in dentistry's contributions to basic research, but my chief concern at this time is for an improvement in, and a greater appreciation of, clinical research in dentistry. This is not to say that dentistry has not made worthwhile contributions in this direction, for, as listed by Kesel,[1] applications of dental research findings have led to many noteworthy accomplishments. These include (1) significant reduction in

* Presented by the incoming president on the occasion of the annual banquet of the International Association for Dental Research, Saturday evening, March 19, 1960.

the incidence of dental caries, largely through fluoridation; (2) judicious use of antibiotics to control oral infections and bacteremias; (3) utilization of calcium hydroxide and other compounds to preserve the vitality of dental pulps; (4) improvement in endodontic therapy; (5) development of sound principles of disinfection and sterilization in dental practices; (6) refinements in the use of anesthetics and analgesics; (7) development of high-speed operative instruments; and (8) development of more efficient dental materials.

Dentistry must continue to improve in its contributions to health measures, and more attention should be placed on clinical research without detracting from basic research. The value of good clinical investigators is well expressed by Fleming,[2] who states that "between the basic research worker and the clinical investigator it is the latter who carries the heavier burden. Not only must he keep up in the sciences but he is also on the 'firing line' where his success and failure are exposed to the bright glare of clinical practice."

Clinical research in dentistry is, in general, more difficult than is true for the field of medicine. The latter assumption is based on the fact that the vast majority of oral diseases are due to multiple etiologic factors, whereas so many of the successes in the field of medicine have been against diseases due to specific microbiologic agents. The difficulties involved should not discourage efforts in clinical research, but they do point up the need for more trained men with a good foundation in research, both basic and applied.

One way of recruiting more clinical researchers is a concentrated effort in the early training of dental students toward an appreciation of research in general and clinical research in particular. This would also develop a greater percentage of dentists capable of critically evaluating clinical research reports. The National Institute of Health support, including the Part-Time Student Fellowship Program, has been a big help toward increasing students' interest in research. Yet, in order to get the maximum results in increasing the numbers of research-oriented students and graduates, we must have more co-operation between basic research workers and dental clinicians.

It is no secret that many clinicians look upon research workers in somewhat the same light as has been described rather facetiously by James H. Scott[3] of England in a discussion on the "Art and Science of Research." According to the latter, some individuals, including clinicians, subscribe to the view that the kind of people who do research are those who are useless at anything else. They are, as a rule, myopic, introverted ectomorphs with poor physiques and second-rate minds. They work in glorified rabbit hutches. Everyday at 11:00 A.M., with much twitching and twittering, they undertake a mass migration to a common room, where they consume tea or coffee and add a few more cells to their lung cancers. They call their work a "project," and it is usually something that has been done 50 years ago by someone who used his brains where they now use an electron microscope or a new staining technic. For them, the ultimate aim of life is to have a paper published in some obscure journal and to see it referred to by a colleague in some slightly less obscure journal.

Now, obviously, we agree with Scott that this is an exaggeration of the present-day scientists in dental research, but I am not so sure but that some clinicians do influence students to think the other way. On the other hand, there are research workers equally as guilty of perpetuating this feud between basic research scientists and dental clini-

cians, by referring to the latter as "tooth carpenters," "plumbers," "mechanics," and certain other very uncomplimentary terms. Of course, most of the bantering between the different groups is good-natured, but unfortunately all of it is not meant as a joke. The worse consequence of the preceding is that many students find it difficult to differentiate between the facetious and the serious meaning intended.

A more realistic criticism of a few clinical teachers is mentioned in the following quotation by O'Rourke and Miner:[4] "Failure to relate clinical problems to scientific methods because of emphasis on vocationalism or worship of what has been called the 'practical' is one of the most retarding influences in dental education. Furthermore, such a failure is actually a criticism of, or at least a reflection on, clinical teaching, because it implies that research methods and a scientific outlook are not essential for proficiency, and that clinical problems can just as well be solved by rule-of-thumb methods."

So far, the importance of good clinical research workers and the need for more dentists trained to evaluate clinical research properly has been discussed. The rest of this presentation will deal with some of the shortcomings of clinical research. In the latter category is the tendency for some investigators to publish too quickly and too often. This is influenced by several factors, including a present-day trend by some individuals and schools to feel that multiple publications are necessary to keep them at the head of the research parade. Another factor is the pressure exerted, at times, by commercial manufacturers on their research staff to aid in getting products on the market quickly and to furnish data for claims of superiority over rival products. This is not to be taken as a blanket condemnation of commercial companies or their research staffs, because I am sure every well-informed individual is aware of their very valuable contributions to both basic and applied research. My plea is for officials responsible for advertising and sales to go more on the sound advice of adequately trained research staffs than on their desire to surpass a rival company in sensational advertising and quick sales.

Scientific evaluation of drugs, both new and old, is of continuing importance in research, and the basic pharmacological and toxicological studies made on animals are only a preliminary step toward determining their real value. Drugs for prophylactic and therapeutic effects on man must, in the final analysis, be tried on humans. Results from such studies on humans are, however, of little value unless carefully controlled and critically analyzed; for, as stated by DeGraff[5] in an editorial comment on clinical research, the literature is full of poorly conceived, uncontrolled studies on many drugs. These uncontrolled studies are often listed as clinical experiences.

It is difficult to determine the real value of drugs in man and especially so when attempts are made to compare relatively similar drugs with each other. In such instances, elaborate and rigidly controlled studies are required to rule out bias. Double-blind tests using placebos and/or other drugs as controls are often used. Even these tests must be carefully controlled as to randomization, representative subjects, adequate numbers, and accurately recorded data; otherwise so-called "double-blind tests" can be worthless.

DeGraff[5] also calls attention to the fact that it is rarely possible to evaluate low-potency drugs as sedatives and tranquilizers in outpatient services where patients are seen only once a week. It is also difficult to evaluate drugs for treatment of gingival

and periodontal diseases unless large numbers of patients are closely followed and rigid control methods are instituted.

As a former member of the American Dental Association, Council on Dental Therapeutics, I have noted that claims for the value of many therapeutic drugs would be based on varying degrees of laboratory evidence, including bacteriological, pharmacological, and toxicological findings. Yet the clinical evidence for these drugs was often based largely or entirely on mere clinical impressions of one or more practitioners. In many of these instances much doubt existed as to whether or not these practitioners had had any previous training in well-planned research methods. A recent symposium on the evaluation of drugs, sponsored by the A.D.A. Council on Dental Therapeutics, dealt with this phase of clinical research, and publication of this conference should be of definite value.

It must be admitted that excellent clinical research in dentistry has been done in the past and that more sound investigators are entering the field, but many more individuals should be adequately trained both as clinical researchers and/or to be able to interpret clinical reports properly.

In conclusion, the following editorial comment by DeGraff[5] seems appropriate: "The clinical investigator is now beginning to achieve his rightful place as a true scientist. His job is hard fraught with many difficulties and pitfalls not known to the scientist working in the seclusion of a laboratory. His reward, after a time-consuming, back-breaking clinical study, is the satisfaction that he has been able to contribute something of real solid value to his fellow man."

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International Association for Dental Research

Abstracts of Papers Presented at the 38th General Meeting

MORRISON HOTEL, CHICAGO, ILLINOIS, MARCH 17–20, 1960

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*Compiled by FRANK J. ORLAND, EDITOR**

I. MICROBIOLOGY I. MARCH 17, AFTERNOON, SECTION A

1. ORAL FLORA IN THE NEWBORN.—*Sumner Joress, Bacteriological Laboratory, New England Medical Center Hospital, and M. Michael Cohen and Marshall B. Kreidberg, Boston Floating Hospital.* It has been assumed by many investigators that the oral cavity of the newborn becomes colonized by bacteria from the mother, nurse, and hospital personnel involved in the care of the infant. Anaerobic organisms have, until recently, been thought to be associated with the development of the gingival crevice in older infants. In a recent study cultures of the oral cavity of 50 newborn infants 1–5 days old were taken. These infants were referred from other hospitals to the Boston Floating Hospital because of surgical, hematological, or infectious complications. The most prevalent organism isolated was *Streptococcus salivarius*, 75 per cent. Other organisms in order of prevalence were coliform bacteria, 25 per cent, staphylococci, 20 per cent, and enterococci, 15 per cent. Almost all the cultures contained more than one organism.

2. MICROBIAL CHANGES AS A RESULT OF FULL-MOUTH EXTRACTION.—*I. L. Shklair and M. A. Mazzarella, Dental Research Facility, Great Lakes, Illinois.* The incidence of oral Lactobacilli, Staphylococci, *Streptococcus salivarius*, and yeast (primarily *Candida albicans*) was studied from nine naval recruits, average age eighteen years, who required full-mouth extraction because of dental decay. Bacterial and yeast counts were made before extraction, during the edentulous period, and after denture insertion. The patients were edentulous for 5–8 weeks. During this period the lactobacilli and yeast were no longer detectable, whereas the *S. salivarius* increased at least twofold in eight of the nine patients. There was no significant change in the *Staphylococcus* population. After dentures were placed, the rise of streptococci was more pronounced. They remained at a high level until the lactobacilli and yeast returned to their pre-extraction level. Once the lactobacilli and yeast were at this level, the *S. Salivarius* also returned to its pre-extraction level. All these population fluctuations occurred within 2–5 weeks, while the incidence of the staphylococci remained unchanged. When the organisms returned to their pre-extraction levels, they did not fluctuate significantly during the period of observation, which lasted 18 weeks after denture insertion. In two cases yeast developed in denture wearers who did not have them previously.

3. INHIBITION OF THE GROWTH OF *Escherichia coli* BY HYDROXYZINE.*—*M. Dworkin and C. E. Smith, Department of Basic Sciences, Indiana University School of Dentistry, Indianapolis, and D. D. Sutton, Institute of Microbiology, Rutgers University, New Brunswick, New Jersey.* The purpose of this investigation was to obtain

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* The members of the Program Committee, Holmes T. Knighton, H. B. G. Robinson, and Dan Y. Burrill, as well as G. W. Ferguson, Program Chairman of the Dental Materials Group, gave invaluable assistance in the preparation of these abstracts.

information relating to the mechanism of action of the tranquilizing drug, hydroxyzine.* At levels of 150 μg/ml it inhibited the growth of *E. coli*. Inhibition lasted for about 8–10 hours, after which there was spontaneous recovery of the cells. Inhibition did not take place in a complex medium and could be reversed in glucose-salts medium by the addition of a variety of substances, e.g., EDTA, amino acid mixtures, organic acids, and magnesium. During the period of inhibition of growth, some of the cells were dying. These cells excreted a heat-stable substance into the medium which could reverse the inhibitory effect of the tranquilizer. It is suggested that the mechanism of action of hydroxyzine involves an interference with the metabolic function of magnesium in *E. coli*.

4. Studies on an Artificial Medium for an Oral Filamentous Organism.—*Patricia H. Beck and Basil G. Bibby, Eastman Dental Dispensary, Rochester, New York.* Preliminary studies were made to determine a chemically defined medium for the oral Gram-positive filamentous organism. Twenty amino acids, ten vitamins, and five nucleic acids were tested for stimulation or inhibition of growth in nutrient agar pour plates. The test agents were supplied to the organism on 6-mm. paper disks, cut from Millipore filter pads and impregnated with water solutions of the test agents. The disks, when dry, were placed on the surface of the pour plates. After incubation the plates were observed for zones of increased or decreased growth around the disks. Preliminary results showed zones of increased growth with asparagine, methionine, threonine, thymine, adenine, uracil, pyridoxine, riboflavin, and biotin. Zones of inhibition were noted with glycine, tyrosine, and guanine. Cystine and glutamic acid showed zones of inhibition where the concentrations were high but increased growth at the outer edges of the zones where the concentrations were lower. Histidine, isoleucine, leucine, lysine, phenylalanine, valine, alanine, xanthine, pryidoxamine, pyridoxal, calcium pantothenate, and *p*-aminobenzoic acid showed no increase or decrease in the amount of growth around the disks.

5. Aerobic and Anaerobic Growth of an Oral Filamentous Micro-organism.—*Marion N. Gilmour, Eastman Dental Dispensary, Rochester, New York.* The filamentous organism, sometimes called *Leptotrichia buccalis* and described as anaerobic, microaerophilic, and aerobic, was examined for these characteristics. Two types of isolates were employed from (*a*) pour plates aerobically incubated and (*b*) streak plates anaerobically incubated. The isolates were tested in serial streakings under aerobic and strictly anaerobic conditions and in small inocula broth tube tests similarly incubated. In the media employed, the isolates fell into three categories; the major number were true facultatives, whereas other isolates were preferentially aerobic or anaerobic, with little, if any, growth occurring anaerobically or aerobically. Those populations which were preferentially aerobic contained 20 per cent or fewer cells which could grow anaerobically.

6. Comparative Study of Anaerobic and Aerobic Oral Filamentous Micro-organisms.—*G. J. Kasai and N. Manelis, University of Chicago, W. G. Zoller Memorial Dental Clinic and the Department of Microbiology.* A comparative study was made of the two groups of oral filamentous micro-organisms in an attempt to clarify the confusion that exists in the literature regarding these forms. A study of their physical characteristics revealed very few differences between the groups. Differences were apparent in their biochemical reactions as well as their physiological pattern. Specific tests were carried out regarding the presence of certain enzyme systems to aid in differentiating the two groups. Other studies also revealed that there were several species within each broad group which did not necessarily belong to the same genera.

7. Intracellular Mineralization by Filamentous Micro-organisms.— *J. Ennever and W. E. Snyder, Division of Dental Research, Procter & Gamble Company,*

* Atarax, J. B. Roerig and Co.

Cincinnati. Filamentous micro-organisms isolated from dentobacterial plaque were grown aerobically and anaerobically on media containing calcium at approximately 1 mg. per cent. At intervals ranging from 2 to 7 days, smears were examined for mineral by the Von Kossa and alizarin red S technics. Those which were positive were further studied by polarized-light transmission through live mounts and by dark-field examination of the residue following incineration at 680° C. The molybdate-phosphate test indicated the salt to be a phosphate. Probably as a result of organic interference, X-ray and electron diffraction analyses were inconclusive. In discussing his demonstration of bone salts in three species of unicellular algae, Pautard, in 1958 stated: ". . . in much lower organisms than has hitherto been suspected there may be a collagen precursor and an inherent power . . . of forming bone-type hydroxyapatite by a process analogous to those found in higher animals." We found that all calcifiable cultures yielded a positive collagen-like response to the Movat method. This work suggests that certain filamentous micro-organisms may be of value as a tool for the study of mineralizing systems.

8. Requirement of Hemin and Vitamin K by Certain Strains of Bacteroides Melaninogenicus.—*Ronald J. Gibbons and John B. Macdonald, Forsyth Dental Infirmary and Harvard School of Dental Medicine, Boston. Bacteroides melaninogenicus* is an anaerobic, Gram-negative, black-pigment–producing rod which is a regular inhabitant of the human oral cavity. Fourteen strains were isolated from the oral cavity of man. Twelve strains were found to require hemin for growth. As little as 1 μg hemin/ml medium was sufficient for maximum growth. Seven strains were also shown to require a diffusable "growth factor" produced by a variety of micro-organisms, including *Staphylococcus aureus.* It was found that menadione and other compounds of the vitamin K group could replace the "growth factor" in the nutrition of the organism. The simplest compound found to satisfy the requirement of the organism was a naphthalene derivative possessing an oxygen, hydroxyl, or carboxyl group in the alpha position. Amino or methyl groups in the alpha position rendered the structure inactive. Substitutions in the 2, 3, or 4 position did not interfere with activity, but substitutions on the second ring inactivated the compounds. Single-ring compounds, such as benzoquinone and hydroquinone, did not support growth. A sole three-membered ring compound tested—alpha methyl anthraquinone—was highly inhibitory.

9. Hydrolysis of Collagen by Cultures of *Bacteroides melaninogenicus.*—*John B. Macdonald and Ronald J. Gibbons, Forsyth Dental Infirmary and Harvard School of Dental Medicine, Boston.* Though evidence of protease activity by members of the human oral microbiota has been presented and attack on native collagen by mixed cultures has been demonstrated, no pure culture from the oral cavity has previously been shown to hydrolyze native collagen. Four organisms from the oral cavity essential in the production of mixed anaerobic infection in the guinea pig were tested for their capacity to hydrolyze collagen. Reconstituted collagen prepared from young guinea-pig skin by extraction in neutral salt (method of Gross) was used as substrate. Of the four organisms, only *B. melaninogenicus* reduced the hydroxyproline content. Forty to 55 per cent of the collagen was hydrolyzed in different experiments. In subsequent experiments it was demonstrated that release of hydroxyproline began after maximum growth of the culture and increased as autolysis occurred. A 48-hour culture of *B. melaninogenicus* was centrifuged, and both supernate and sedimented cells were tested for their activity against gelatin viscosimetrically. Most of the activity was associated with the cells. It was not increased by lysing. Lysed cells of *B. melaninogenicus* were incubated with fresh sterile untreated rat tail at 37° C. for 4 days. Hydroxyproline was released from these preparations but not from heat-inactivated controls.

10. Growth of *Treponema microdentium* on Surface of Blood Agar Plates.—*Sigmund S. Socransky and John B. Macdonald, Forsyth Dental Infirmary and Harvard School of Dental Medicine, Boston.* Isolated spirochete colonies were successfully

grown on the surface of blood agar plates from both pure and mixed inocula. The medium employed was a veal heart infusion base containing 1.5 or 3 per cent agar and 10 per cent defibrinated rabbit blood with or without 10 per cent ascitic fluid. All plates were stored in Brewer jars containing 95 per cent hydrogen and 5 per cent carbon dioxide for 24 hours before use. The manipulation of inocula was carried out in an isolation chamber* in which the air had been replaced by nitrogen. Continuous polarographic determination of the atmosphere in the chamber demonstrated that the concentration of oxygen did not rise above 0.05 per cent. Five strains of *Treponema microdentium* were streaked on plates and then sealed in Brewer jars, the whole operation being performed in the nitrogen chamber. As controls, two more sets of plates were streaked in air, using the same sources as inocula. One set was immediately sealed in a Brewer jar and the other exposed to air for an additional 30 minutes. All three sets of plates were incubated for 5 days in Brewer jars containing 95 per cent hydrogen and 5 per cent carbon dioxide. All five strains of spirochetes grew abundantly when streaked inside the chamber. Little or no growth occurred on the "bench-streaked" controls. Single isolated colonies from the "chamber-streaked" plates could be passed successfully in the chamber from plate to plate or plate to tube. In seventeen trials with mixed inocula, large numbers of spirochete colonies grew on the agar of plates streaked in the chamber. No isolated spirochete colonies grew on similar "bench-streaked" material.

11. *Candida albicans* IN THE SALIVA OF DIABETICS.—*I. W. Weinstein, L. B. Duke, R. S. Peters, and A. N. Bahn, Research Veterans Administration Hospital and Northwestern University Dental School, Chicago.* In preliminary studies *Candida albicans* was isolated from the saliva of approximately 80 per cent of thirty-six patients with diagnosed diabetes mellitus. In contrast, this yeast was isolated from the salivas of approximately 50 per cent of thirty-six patients with no symptoms of diabetes. Stimulated saliva samples were plated on Sabouraud's dextrose agar and corn-meal agar. The isolated yeasts were then identified by their production of chlamydospores on Liu-Newton agar, zein agar, and Nickerson-Mankowski agar media. Colonial morphology of the yeasts on Levine's eosin methylene blue agar was also used in the identification. Quantitative studies of the numbers of *C. albicans* in the salivas of diabetics and non-diabetics are now in progress. Appropriate dilutions of fresh saliva are filtered through a HA millipore filter. The filter pad is placed on the above-mentioned media, and a count of the yeasts is obtained. In this manner a comparison of the numbers of *C. albicans* in the saliva of diabetics with the numbers in the saliva of non-diabetics is made.

12. INCIDENCE AND ECOLOGY OF ORAL PROTOZOA.—*Wayne Warde Wantland and Edna Marie Wantland, Illinois Wesleyan University, Bloomington.* Direct microscopic examination and culturing of samples taken from the mouths of 500 persons of various ages revealed the following incidences of mouth Protozoa: *Entamoeba gingivalis,* 39.8 per cent; *Trichomonas tenax,* 22 per cent; both *E. gingivalis* and *T. tenax,* 17.2 per cent. While both protozoans were found not infrequently in mouths rated clean and healthy, *E. gingivalis* was demonstrated invariably and *T. tenax* frequently in cases of progressive and advanced periodontosis. The accumulation of heavy calculi on teeth was highly indicative of the presence of both *E. gingivalis* and *T. tenax.* Both protozoans were found most frequently in persons between thirty and fifty years of age and least frequently in young children and college students. The degree of salivary flow was not correlated in any way with the presence or absence of Protozoa. Comparison of samples of saliva from persons harboring large numbers of oral Protozoa (active trophozoites) with saliva from persons negative for these organisms revealed many more degenerated epithelial cells and leukocytes, as well as more bacteria-covered cells in the former. *Entamoeba gingivalis* was capable of cytolysis of leukocytes and erythrocytes, as well as

* Kewaunee C.B.R.

engulfment of leukocytic nuclear fragments and whole erythrocytes. Optimum pH for growth and reproduction in cultures and in mouth habitat was 7.6–7.8. Both *E. gingivalis* and *T. tenax* were successfully cultured in either Balamuth's egg-yolk fluid medium or St. John's overlaying Dobell and Laidlaw's egg slants. Near-axenic cultures and encystment of *E. gingivalis* were promoted by replacing the fluid medium in culture vials every other day, thus diminishing the number of bacteria. Refrigeration at 2° C. promoted encystment of *T. tenax*. In cultures containing both protozoans a definite alternation of ecological ascendancy was frequently observed.

II. EMBRYOLOGY AND HISTOLOGY. MARCH 17, AFTERNOON, SECTION B

13. PROTECTIVE EFFECT OF CYSTEINE AGAINST AMINOACETONITRILE (AAN)-INDUCED DEFORMITIES IN CHICK EMBRYOS.—*S. Jerome Zackin and Paul Goldhaber, Harvard School of Dental Medicine, Boston.* In a previous study it was shown that the injection of 1.0 mg. of a lathyrogenic agent, aminoacetonitrile (AAN), into the yolk sac of 6-day chick embryos induces hypogenesis of the mandible or twisting of the beak, plus deformities of the tibiotarsal region, in all embryos surviving to the thirteenth day. Reducing the dose of AAN to 0.7 mg. resulted in deformities in 70 per cent (59 of 84) of the embryos. Since others have shown some degree of protection from the effects of lathyrogenic agents in rats by the use of various amino acids, an attempt was made to test the effectiveness of a number of these agents in the chick embryo by simultaneous injection of the amino acid (or acids) with AAN. When 8.0 mg. of cysteine were administered in conjunction with 0.7 mg. AAN, only 33 per cent (14 of 42) of the embryos were deformed. Administration of cysteine (8.0 mg.) plus glutamine (8.0 mg.) with AAN (0.7 mg.) resulted in 9.5 per cent (2 of 21) deformities. Preliminary data indicate that glutathione affords some protection as well. On the other hand, cystine, methionine, serine, glutamine, and glutamic acid were without effect. Subsequent experiments suggest an in vitro interaction of cysteine and AAN, rather than an in vivo inhibition or reversal of the teratogenic effects of AAN, since injection of cysteine and AAN at separate sites—yolk sac and chorioallantoic membrane—did not reduce the number of AAN-induced deformities. Further investigations are in progress in an attempt to determine the mechanism of the protective effect.

14. MATERNAL TRANSMISSION OF TETRACYCLINE TO THE SKELETON OF THE DEVELOPING EMBRYO.— *Gerrit Bevelander and Gloria K. Rolle, New York University.* Previous studies (Bevelander, Nakahara, and Rolle, 1959; Bevelander, Goldberg, and Nakahara, 1959) have shown that injection of tetracycline in the yolk sac of the developing chick embryo and exposure to tetracycline by the echinoderm embryo resulted in inhibition of skeletal development and retention of a fluorophore in the reduced mineralized portions which were deposited. Additional experiments to be reported are concerned with the effect of feeding tetracycline to the laying hen, the incubation of the eggs, and examination of the embryos for the presence of the drug. The embryos were removed at 4 days and daily thereafter for 10 successive days. Gross and microscopic examination of the embryos revealed the presence of tetracycline fluorophore in the skeletal system from the ninth to the fourteenth day, when the experiments were terminated. These experiments clearly demonstrate that the drug passes from the intestinal tract of the mother to the egg. It is then incorporated and retained in the skeletal system of the developing embryo.

15. THE EFFECT OF UTERINE CIRCULATORY ARREST ON THE DEVELOPMENT OF THE RAT FETUS.—*Lee E. Feild, Seymour J. Kreshover, and Jacob E. Lieberman, National Institute of Dental Research, National Institutes of Health, Bethesda.* The effects of temporary anoxia upon fetal mortality and upon the frequency of congenital deformities, including cleft palate, were studied. Anoxia was induced by a temporary arrest of the maternal blood supply to one uterine horn in each of 249 gravid Sprague-Dawley rats for periods of 10–60 minutes during stages of pregnancy from 7 to 17

days. Developmental anomalies were observed only in the fetuses of 169 rats treated between the twelfth and fifteenth days of gestation. Twenty-sixth of the 507 viable experimental young in this group were deformed, whereas two of the 523 viable control young were deformed. The greatest frequency of deformities (6 out of 28, or 17.6 per cent) occurred after a 45-minute vascular occlusion on the fifteenth day. There were 13 instances of palatal clefts, 8 of which occurred in young subjected to anoxia on the fourteenth day. There were 18 young with limb defects or other abnormalities, 5 of which also had cleft palates. It was also possible to study the relationship of vascular occlusion to frequency of maceration and resorption. There was a highly significant statistical difference ($P < 0.01$) between the average proportions of maceration and resorption in the experimental and control uterine horns of the 249 rats. This proportion in the experimental horns increased as the duration of circulatory arrest increased. It was apparent that the thirteenth day is the most critical for fetuses in the experimental horns, there being a sharp rise in the maceration-resorption frequency from the seventh to the thirteenth day and then a moderate decline to the seventeenth day.

16. The Establishment of Individual Odontogenic Areas in Human Embryos.—*C. H. Tonge, King's College, Newcastle upon Tyne, England.* The early differentiation of the oral epithelium and the arrangement of the underlying mesenchymal condensation beneath it were studied in a graded range of human embryos to ascertain the formation of specific odontogenic areas. The range of specimens was made up of human embryos cut sagittally, coronally, and transversely and belonging to Streeter horizons XIII–XXIII, covering the period of 27–48 days' ovulation age. At 28–30 days, low columnar epithelium lined the oral cavity, the underlying mesenchyme comprising densely packed stellate or fusiform cells. Epithelial proliferation regarded as potentially odontogenic could be identified in the premaxillary and mandibular regions in embryos of 31–32 days' ovulation age. The differentiation of specific odontogenic areas for individual teeth preceded the separation of a lip and was observed in embryos of 36–38 days. The mesenchymal condensation is an essential part of the developing odontogenic area and was clearly differentiated from other mesenchymal condensations. It further differentiated into a dental papilla and an inner layer of the dental follicle. In the region between adjacent odontogenic areas the epithelium was less proliferative, and the mesenchymal response not so pronouncd. The epithelium of each odontogenic area had an individual pattern identified by reference to its shape and size. Later the invagination of the odontogenic epithelium and the formation of the dental lamina were associated with a clearer definition of individual tooth pattern and were observed in embryos of 46–48 days' ovulation age.

17. Stages in the Prenatal Development of the Human Bony Palate.—*Bertram S. Kraus, Department of Orthodontics, School of Dentistry, University of Washington, Seattle.* There has been scant and inadequate study of the changing morphological relationships achieved by the premaxillae, maxillae, and palatine bones as they develop from ossification centers to the eventual formation of the bony palate as known in postnatal life. In an attempt to describe the normal morphological sequence of events that takes place, a series of 151 fetuses ranging in age from 7 to 18 weeks was cleared and stained with alizarin red S. Study of the stained bony palate was conducted by microscopic examination, by palpation, by measurement, and by photography. It was found that seven morphological stages in development could be described. Allometric studies indicated that the bony palate achieves its normal, characteristically human proportions through rigidly controlled, though highly complex, patterns of acceleration and deceleration of growth in the various bony components of the palate.

18. Mucopolysaccharides of Tissue-cultured Human Amniotic Cells and Chick Chorioallantoic Membrane.—*Herman H. Langkamp, Rodger F. Sisca, and*

John C. Thonard, School of Dentistry, University of Pittsburgh. If depolymerization by mucopolysaccharases of the intercellular cementing substance is a factor in the initiation of chronic marginal gingivitis, it becomes important to know whether this action can occur per se in the absence of other oral environmental factors. The present work reports on the use of epithelial sheets grown in tissue culture conditions and of chorioallantoic membranes of chick embryos as test systems for the *de novo* action of mucopolysaccharases on epithelial intercellular cementing substance. Transfers of human amniotic cell cultures were made into Leighton tubes containing glass cover slips and 1-ml. standard tissue culture medium. The initial concentration of cells was 25,000. Cell cultures 14 days old, in which the medium had been changed every 2 days, were selected because of the good contiguity of the monolayers at this time. The cover slips and attached monolayers were removed, fixed in 4 per cent formalin–80 per cent ethyl alcohol, and stained with Hale's stain (iron absorption), periodic acid–schiff (P.A.S.) technics, alcian blue, Giemsa and toluidine blue at pH's of 6.0 and 2.5. Chorioallantoic membranes were dissected from 14–16-day-old chick embryos, fixed in either 10 per cent formal saline or 4 per cent formalin–80 per cent ethyl alcohol, paraffin-imbedded, and sections stained by the above technics. Results showed that human amniotic cells grown in tissue culture and chick chorioallantoic membranes contained mucopolysaccharides in the epithelial intercellular cementing substance as demonstrated by specific histochemical technics, in agreement with previous findings on human gingival epithelium. Experiments testing the action of bacterial and animal polysaccharases under sterile conditions on these substrates are being pursued.

19. The Effect of Hypoxia on Developing Enamel of Neonatal Rats.—*William F. Via, Jr., and William K. Elwood, Henry Ford Hospital, Detroit.* Previous studies have shown a relationship between a poor respiratory response of the newborn human and the occurrence of enamel hypoplasia. This experiment was designed to determine whether artificially induced hypoxia in neonatal rats (approximately 24 hours old) would produce a disruption of amelogenesis. Litters of newborn rats were divided into control and experimental groups. The experimental ratlets were subjected to a mixture of 95 per cent N_2 and 5 per cent CO_2 in a bell jar until they were unconscious and the respiratory rate was less than 2 per minute. Thirty to 80 minutes were required, compared with 12–15 minutes for adult animals. The control littermates were exposed to the same conditions except that they were allowed to breathe room air. Both groups were killed on the fourth, tenth, or thirty-first day following the experiment. Celloidin serial sections were made of the heads. No defects were found in the developing enamel of the ratlets in litters exposed to the asphyxiant for 30, 40, 55, or 60 minutes. However, in one group exposed for 40 minutes, 4 died, and the remainder had enamel defects in both control and experimental ratlets, indicating that conditions other than hypoxia were responsible for the defects. Those animals exposed for 50, 75, and 80 minutes had enamel defects, and a greater number of deaths from asphyxia occurred among these groups. The results suggest that, while the newborn is more resistant to hypoxia than the adult, a prolonged asphyxial insult may produce a disruption of amelogenesis.

20. The Effect of Manganese Chloride on Amelogenesis.—*William K. Elwood, Henry Ford Hospital, Detroit.* Enamel hypoplastic defects are consistently produced on the incisors of rats by the subcutaneous administration of 400 mg $MnCl_2 \cdot 4H_2O$/kg body weight. In this study, guinea pigs and hamsters were injected with various dosages of $MnCl_2 \cdot 4H_2O$, and the incisors were observed for the development of enamel defects.

The guinea-pig group consisted of twelve experimental animals, two NaCl-injected controls, and two uninjected controls. The experimental animals were injected subcutaneously with dosages of $MnCl_2 \cdot 4H_2O$ ranging from 150 to 490 mg/kg body weight. Dosages of 400 mg $MnCl_2 \cdot 4H_2O$/kg body weight and higher were usually fatal for the guinea pig as compared with 800 mg/kg for the rat. All surviving ex-

perimental guinea pigs developed gross hypoplastic enamel faults. One NaCl control, which survived a biopsy procedure 26 days postinjection for the removal of a granulomatous lesion, also developed enamel defects. The NaCl injection was ruled out as being responsible for the enamel defects in the biopsied animal by injecting a group of eight guinea pigs with heavy dosages of NaCl and observing no similar faults. Hamsters injected subcutaneously with 150–400 mg $MnCl_2 \cdot 4H_2O$/kg body weight were examined periodically for 2 months. No hypoplastic enamel lesions occurred. Four control hamsters also showed no enamel defects. It is concluded that amelogenesis in the guinea pig and hamster is not affected by the same range of dosages of manganese chloride based on body weight as is the rat.

21. Electron-microscopic Study of Terminal Bar Systems in Ameloblasts.—*Marie U. Nylen and David B. Scott, National Institute of Dental Research, National Institutes of Health, Bethesda.* Terminal bars have been interpreted optically as concentrations of dense intercellular cementing material. Following electron-microscopic investigations of various tissues, this term has needed revision, and terminal bars are now defined as thickened areas of adjacent cell membranes with an accumulation of dense material in the subjacent cytoplasm. As such, they are comparable to the desmosomes in structure but differ from them in shape and dimension. In view of these findings, a re-evaluation of the so-called terminal bar systems of the ameloblasts was considered important. In a study of thin sections from the continuously growing incisors of 4-day-old mice a terminal bar system, as defined above, was found present in the basal ends of the ameloblasts, i.e., toward the stratum intermedium. These terminal bars first appeared following the mitotic stage, before either dentin or enamel matrix elaboration had begun. On the other hand, a similar structure was not observed in the distal ends of the ameloblasts, at the site of the terminal bar system which has been described optically and related to enamel matrix formation. Instead, accumulations of dense material were seen, but they were located between the cells, resembled young enamel matrix morphologically, and were present only during active matrix formation. Although these structures may resemble terminal bars optically, they cannot be identified as such at the electron-microscopic level. They may be most simply explained as being a stage in the production of enamel matrix. These observations suggest that the term "terminal bars" should be restricted to the structure observed in the basal ends of the cells.

22. Nitro BT Formazan Disclosure of Oxidative Enzymes in Oral Tissues.—*Haritun A. Shahrik, Bertram Eichel, and Vincent F. Lisanti, Institute of Stomatological Research, Sias Research Laboratories, Brooks Hospital, Brookline, Massachusetts.* The Nitro BT formazan technic of Nachlas and associates was modified and employed for the histochemical disclosure of endogenous reducing enzyme (ERE) activity and several oxidation-reduction enzymes in oral tissues. Modifications in technique included (1) changes in method of incubation, (2) sequential omission of the components of each enzyme reaction mixture, and (3) changes in the method of fixing and mounting of tissue sections. ERE, succinic dehydrogenase (SD), DPNH reductase (DPNHR), and glucose-6-PO_4 dehydrogenase (G6PD) activities were demonstrated in human tooth pulp of freshly extracted non-carious teeth. The enzyme activities were predominantly localized in the odontoblast layer, nerves, blood vessel walls, and to some extent in isolated cells within the connective tissue. Purple formazan production as a measure of G6PD activity was considerably less intense when compared with that for SD and DPNHR activities. Purple Nitro BT formazan as a measure of SD and DPNHR was also evident in the mucosa and submucosa of human gingiva specimens. The formazan darkly stained the basal-cell layer of the gingiva. The intensity of the stain decreased as the superficial layers of epithelium were approached. ERE activity in the gingival epithelium was accompanied by pinkish-violet staining and was most pronounced in the direction of the superficial epithelial cells. This may have reflected the presence of sulfhydryl-containing compounds. Hamster

and rabbit tongue mucosa SD activity was also disclosed by purple formazan deposition. The characteristic blue formazan deposits reported by Nachlas *et al.* were identified only in the tongue striated muscle. The latter stain was exceedingly intense and reflected the very high SD activity in this tissue.

23. DESMOSOMES, TONOFIBRILS, MITOCHONDRIA—METABOLIC PARTICULATES OF HUMAN GINGIVAL EPITHELIAL CELLS.—*J. A. Yacovone, V. F. Lisanti, H. A. Shahrik, and B. Eichel, Institute of Stomatological Research, Sias Research Laboratories, Brooks Hospital, Brookline, Massachusetts.* Succinic dehydrogenase (SD) activity has been disclosed in human gingival epithelial cells and submucosa cell components by means of modification of the Nitro BT formazan method. Microscopic examination of the prickle cells under oil immersion disclosed the following details. The desmosomes appeared as dark purple oblate spherioids between the cells. The tonofibrils were less intensely stained and gave the impression of a fingerprint pattern. The mitochondria stained intense purple and were concentrated at either end of the eliptical cell nucleus, suggesting polarity of the nucleus. A thin layer of mitochondria could be seen along the periphery of the nucleus in its narrow diameter. The tonofibrils appeared to run from the desmosomes inward to the cytoplasmic mass containing the mitochondria around the nucleus. These structures did not stain in the absence of succinate. The latter implies localization of SD in the mitochondria, the desmosomes, and the tonofibrils.

24. THE HISTOLOGIC EFFECTS OF DL-ETHIONINE ADMINISTRATION.—*Samuel M. Standish, Indiana University School of Dentistry, Indianapolis.* A series of pilot studies was designed to determine the histologic effects of DL-ethionine administration on various organ systems and particularly the salivary glands in Sprague-Dawley rats and C57/BL7 mice of both sexes. The ethionine was administered with a normal laboratory diet, a protein-deficient diet, by I.P. injection, and by both diet and injection. Dosages in the diet were 0.25, 0.5, and 1.0, and injection dosages were 25 mg. daily or 50 mg. every other day. Sacrifice was made at various sacrifice intervals ranging from 5 to 60 days. The tissues examined microscopically were the liver, pancreas, salivary glands, spleen, lymph nodes, intestine, thyroid, kidney, adrenal, and ovary or testis. The most consistent histologic changes were noted in the liver, with the alterations ranging from mild fatty metamorphosis to severe alterations in the size and shape of the hepatic cells, suggestive of hepatoma. Fatty livers were observed most frequently in female animals, male animals being refractory to liver change except in protein deficiency. Specific morphologic changes were not consistently observed in all groups in the pancreas or salivary glands, although testicular atrophy and associated changes were noted at 14 days.

III. PHARMACOLOGICAL AND CHEMICAL INVESTIGATIONS. MARCH 17, AFTERNOON, SECTION C

25. INTRAVENOUS TOXICITY OF LOCAL ANESTHETICS IN MICE.—*Donald A. Wallace, Erica V. Friederici, and Om P. Gupta, University of Illinois College of Dentistry, Chicago.* Extemporaneously prepared local anesthetic solutions were injected intravenously at a steady rate over an interval of 10 seconds. The dose approximated the LD_{50} value in all experiments. Thus the rate of injection depended on the volume, which, in turn, depended on the weight of the mouse and the LD_{50} value. Procaine HCl in 0.5, 1, 2, and 4 per cent aqueous solution killed approximately the same proportion of the animals tested under these conditions. Lidocaine HCl in 0.5, 1, and 2 per cent aqueous solutions behaved similarly. At the 0.25 per cent level, lidocaine HCl was less toxic, but perhaps not significantly so. Addition of epinephrine (1:50,000) to a 2 per cent aqueous solution of procaine HCl resulted in an increase in toxicity. Using procaine HCl as a standard, relative lethal toxicity values were determined for various mixtures of procaine HCl with butethamine HCl and propoxycaine HCl. The lethal effects were found to be additive. Neither potentiation nor antagonism was observed.

26. Gingival Response to Systemic Administration of Diphenylhydantoin Sodium in Cats.—*Jun Ishikawa and Irving Glickman, Tufts University School of Dental Medicine, Boston.* The response of the gingiva to the systemic administration of diphenylhydantoin sodium* was studied clinically and microscopically in 19 cats. Seven mg. of diphenylhydantoin sodium per kilogram of body weight were fed daily by mouth in a capsule to 16 of the animals. Three animals served as controls. Marginal erythema and some enlargement of the gingiva occurred in 14 of the experimental animals. Gingival hyperplasia occurred in 8 animals, appearing 1–2 weeks after the drug was administered. Clinically, the hyperplasia took the form of vascular beadlike masses which extruded from the space between the gingival margin and the tooth, diminishing in redness as time progressed. These masses presented distinguishing microscopic features which differentiated them from the non-hyperplastic gingiva. These included prominent proliferation of the connective tissue cells and endothelium to form numerous new blood vessels and proliferation of the epithelium with acanthosis and elongated rete pegs. Inflammation was present in both the hyperplastic and the non-hyperplastic gingiva.

27. Parotid Fluid-free 17-Hydroxycorticosteroid Concentration as an Indicator of Adrenocortical Status.—*John R. Prigmore and Ira L. Shannon, School of Aviation Medicine, USAF, Brooks Air Force Base, Texas.* Preliminary studies involving the oral administration of synthetic analogues of hydrocortisone have indicated that non-conjugated 17-OHCS concentration changes in parotid fluid could provide a sensitive index of adrenocortical function. To test this concept further, alterations in body-fluid steroid levels resulting from experimental conditions designed to produce adrenocortical stimulation or depression were studied. Under the influence of a 2-hour intravenous infusion of 40 I.U. ACTH or the intramuscular injection of 40 I.U. ACTH, altered parotid fluid steroid levels in healthy young male adults closely paralleled increases seen in serum and urine 17-OHCS concentration. A decreased steroid concentration in parotid fluid, serum, and urine followed the oral administration of a single dose of 50 mg. of Triamcinolone (delta-1, 9-alpha-fluoro, 16-hydroxy hydrocortisone). This preparation, since it is not detectable by the phenylhydrazine color reaction employed, produces a drop in measurable 17-OHCS by suppression of endogenous adrenocortical secretion. These findings further solidify the concept that steroid levels in parotid fluid can be used as an indicator of adrenocortical status.

28. The Effect of Hydrocortisone on Attrition and Extrusion Rates of Rat Incisors.—*Frank Sobkowski and Melvin Smithgall, Department of Physiology, University of Pennsylvania, Philadelphia.* Thirty-five male albino Wistar rats of approximately the same age (10 weeks) and the same weight (90–100 gm.) were divided into five equal groups. During a period of 15 days the extrusion and attrition rates of the maxillary incisors were recorded at 5-day intervals for control purposes. Daily food intake was also measured. Tap water was used as the drinking solution. The animals were individually caged in a room that was maintained at constant temperature and humidity. On day 15, daily intramuscular administration of specific doses of hydrocortisone was begun. The following doses were used: Group I, 1.0 mg.; Group II, 2.0 mg.; Group III, 4.0 mg.; Group IV, 8.0 mg.; and Group V, 0.2 cc. physiologic saline. On the eleventh day following the initial hydrocortisone administration, all animals were weighed, sacrificed, and autopsied. The right adrenal gland was then removed, dissected free of its surrounding fat, and weighed. Daily administration of hydrocortisone caused a significant increase in the extrusion rate within 5 days, but a dose response was not evident until the second recording time at day 10. The effect on the attrition rate was not consistent throughout the administration period. Food intake was significantly decreased during the administration of the hormone, with the larger doses resulting in a greater effect than the smaller doses. All hormone-injected groups displayed a significant decrease in adrenal weight.

* Dilantin Sodium Kapseals, Parke, Davis and Co.

29. Blood Serum Total 17-Hydroxycorticoids Levels in Necrotizing Ulcerative Gingivitis.—*R. H. Loving, Periodontic Department, Dental Clinic, T. B. Weber and M. A. Mazzarella, Dental Research Facility, Great Lakes, Illinois.* A multitude of causes have been implicated as contributing factors for the development of acute necrotizing ulcerative gingivitis. Prominent among them have been emotional effects which may elicit an endocrine response detectable by steroid change. The present study was undertaken to seek relationships between the clinical picture and stress, as measured by the total 17OHCS levels in blood serum. To determine whether or not any association existed, naval recruits were studied while in the acute phase and after the remission of these acute manifestations. An additional group with healthy periodontium was studied, in order to establish the total 17OHCS levels in the serum of normal patients. Fifty naval recruits, seventeen to nineteen years of age, were studied in their first weeks of basic training. Total 17OHCS determinations were made on the serum of each patient by the *n*-butanol extraction procedures of Reddy, as modified by Prigmore, Shannon, and Feller. The normal group presented levels with a mean value of 46.4 μg/100 ml. The clinically acute group showed a significantly different average value of 136.6 μg/100 ml. After acute clinical manifestations had subsided, these levels dropped to an average group value of 73.1 μg/100 ml. A relationship was found to exist between acute necrotizing ulcerative gingivitis and stress factors as measured by the total 17OHCS levels in blood serum of naval recruits.

30. Skin and Oral Mucosal Response to Single Vitamin A Injection.—*Lionel Gold, Alvin L. Morris, and Harvey G. Seligman, Dental School, University of Pennsylvania, Philadelphia.* Jewell *et al.* found that a single 0.03-cc. intracutaneous injection of vitamin A palmitate solution in the rabbit caused marked thickening of the epidermis, excluding corneum (*Proc. Soc. Exptl. Biol. Med.*, **96**:162, 1957). Experiments were designed to test the effect of high local concentrations of vitamin A on oral mucosa. Six hamsters were given a single 0.02-cc. intramucosal injection of vitamin A palmitate solution (50,000 U.S.P. units/cc suspended in vehicle) in the right buccal pouch and 0.02 cc. of vehicle in left pouch. A second group of six hamsters received applications of a vitamin A ointment (500,000 units/ounce) to the right pouch and vehicle to left pouch twice a day for 5 days. Animals were sacrificed at 2, 4, 7, 14, and 21 days. Histologic sections were prepared and stained with Rematoxylin and eosin. Examination of the sections showed no oral epithelial response. A group of three rabbits, three guinea pigs, and five hamsters was used in another experiment, in which vitamin A solution and vehicle were injected in separated sites both intracutaneously (0.3–0.6 cc.) and intramucosally (0.1–0.2 cc.). All animals were sacrificed at 7 days. Histologic sections of experimental and control tissues were prepared and stained with hematoxylin and eosin. The epidermal changes as described by Jewell *et al.* were uniformly reproduced in all animals. No oral mucosal changes were noted. In contrast to skin, the oral mucosa of rabbits, hamsters, and guinea pigs appears to be unaffected by high local concentrations of vitamin A.

31. Determination of Quaternary Compounds in the Presence of Sodium Nitrite.—*Henry M. Koehler and John J. Hefferren, American Dental Association, Chicago, and Edward G. Feldmann, American Pharmaceutical Association, Washington, D.C.* Solutions of quaternary ammonium compounds which are marketed to the dental profession for the chemical disinfection of instruments frequently contain sodium nitrite as a rust inhibitor. The assay method which is official for benzalkonium chloride and benzethonium chloride and has been successfully applied to other quaternary ammonium compounds involves the reaction of the quaternary amine with potassium ferricyanide followed by the determination of the excess reagent, using a standard iodometric titration. Since sodium nitrite and other agents capable of undergoing oxidation-reduction reactions cause interference in the official assay, the following method was developed to circumvent this interference. A volume of quaternary ammonium concentrate was diluted with suitable solvents, the pH adjusted with a buffer,

and the solution treated with excess potassium ferricyanide solution. The yellow precipitate was collected, washed with water, dried, and weighed. This precipitate could then be titrated in non-aqueous solvents with perchloric acid to determine the number of equivalents of quaternary amine in the complex. The gravimetric modification of the official method and the subsequent titrimetric procedure are complementary, especially with those quarternary ammonium compounds that are not single compounds but rather a homologous series of quaternary amines with alkyl chains of different lengths, e.g., C_8–C_{18}. Sodium nitrite, dyes, and other salts commonly found in these preparations do not ordinarily interfere.

32. Comparative Fixation of Calcium and Strontium by Synthetic Hydroxyapatite.—*R. C. Likins, H. G. McCann, and A. S. Posner, National Institute of Dental Research, National Institutes of Health, Bethesda, and the American Dental Association Research Division, National Bureau of Standards, Washington, D.C.* Four samples of calcium hydroxyapatite were prepared by precipitation from solutions containing added Ca^{45} and carrier-free Sr^{89}. The method of synthesis was varied to yield precipitates of different crystal size. By X-ray diffraction or electron microscopy the preparations were found to have average crystal sizes (*c*-axis direction) of 180 A, 280 A, 1.5 μ, and 4.0 μ. The Sr^{89}/Ca^{45} ratio of these precipitates, relative to the solution at the start, was 0.968, 0.924, 0.597, and 0.591, respectively. After exchange in 0.01 M calcium acetate to remove surface Ca^{45} and Sr^{89}, the Sr^{89}/Ca^{45} ratio of the remaining solid was 0.897, 0.843, 0.596, and 0.591, respectively. The results show a discrimination against strontium in crystal formation, which increased with crystal size. The data suggest that the reported preferential release of skeletally deposited strontium may be due to perfection of newly formed bone crystals.

33. Synthetic Carbonate Apatite.—*Otto R. Trautz and Racquel R. Zapanta, New York University College of Dentistry.* By treating amorphous calcium carbonate phosphate precipitates in the autoclave at 360° C. and 200 atm. steam pressure, we obtained crystalline materials whose X-ray diffraction patterns are those of an apatite with a comparatively short *a*-axis, while the *c*-axis is somewhat longer than that in hydroxyl apatite. All naturally occurring (mineral) carbonate apatites, i.e., the francolites (carbonate flour apatites) and the dahllites (carbonate hydroxyl apatites), had shorter *a*-axes than did the corresponding carbonate-free apatites. While the precipitates from neutral aqueous solutions of mixed carbonate and phosphate may contain considerable amounts of calcium carbonate co-precipitated with the calcium phosphate, we never observed a shortening of the *a*-axes in these products. Neither did the biological apatites in bone, dentin, and enamel exhibit the shortened *a*-axes. We used the shortened *a*-axes as the criterion in differentiating a carbonate apatite from a mixture of amorphous carbonate and apatite and classified our autoclave products as dahllites. Their *a*-axes were as short as those in the best mineral dahllites. Heating the amorphous precipitate in 60 per cent KOH at 100° C. also produced dahllite.

34. Alizarin Red S Adsorption by Apatite.—*Howard M. Myers, University of California, San Francisco.* A colorimetric analysis of alizarin red S uptake by synthetic apatite (Molar Ca/P 1.5, density 3.12) was undertaken. The data indicated that sintering reduced dye uptake much less than it reduced the specific surface of the apatite. A flat orientation of the dye molecule on the surface of apatite was consistent with the adsorption isotherm obtained. Chelation of calcium by the ligand dye molecule apparently did not occur at pH values below 8.5. The pH was without significant effect on dye uptake at values lower than 8.5. Phosphate ion interfered with dye adsorption. Arsenate interference, while present, was much less than that of phosphate. Replacement of hydroxyl ions of apatite by fluoride reduced alizarin red S uptake. Cation replacement of calcium had an effect on dye binding only when sodium was the exchanging ion.

35. THE INCORPORATION OF PROLINE C^{14} INTO COLLAGEN PROLINE AND HYDROXYPROLINE.—*Ernest Hausman, Department of Dental Research, University of Rochester School of Medicine and Dentistry, Rochester, New York.* Previous investigators have attempted to obtain information on the mechanism of hydroxylation of proline by determining the specific activity ratios of collagen-incorporated hydroxyproline/proline after administration of proline C^{14}. In the present study proline C^{14} was administered to guinea pigs which were sacrificed at 3-hour intervals during a 24-hour period; 0.45 M NaCl-soluble collagen was extracted from skin, purified by the method of J. Gross (*J. Exptl. Med.*, **107**:247, 1958), hydrolyzed, and the specific activity of radiochemically pure samples of proline and hydroxyproline determined. The hydroxyproline/proline specific-activity ratios were in the range of 0.9–1.1. The standard error of the method in determining specific activities of proline and hydroxyproline was 7.5 and 8.5 per cent, so that the above ratios are not significantly different from 1. The in vitro addition of proline C^{14} to guinea-pig skin at the first collagen-extraction step resulted in insignificant radioactivity in the collagen. No difference in proline-specific activity was obtained from collagen aliquots acid-hydrolyzed and others previously incubated with highly purified collagenase (N. Grant, *Arch. Biochem. Biophys.*, **82**:245, 1959) and ultrafiltered. The variation of these results with those of previous investigators (N. Green *et al., Biochem. J.*, **71**:55, 1959, and W. Robertson, *J. Biol. Chem.*, **234**:105, 1959) may be attributed to the latter lack of sufficient controls.

36. THE STERILIZATION OF SALIVA.—*Frederick W. Kraus and Clara J. Williams, Veterans Administration Hospital, Birmingham, Alabama.* Several sterilizing procedures were evaluated according to their success in producing sterile saliva consistently and according to their denaturing effects. Four criteria were selected to determine the extent of destruction. They were the concentrations of peroxidase, catalase, amylase, and antigenic materials reacting with antihuman precipitin serum.

Among chemical methods, 3 per cent ethylene oxide acting for 4 hours at 37° C. sterilized 84 per cent of the samples and preserved the normal ranges of the criteria. Five per cent hydrogen peroxide produced sterility after 24 hours at 4°–37° C. but destroyed two of the enzymes. Among physical methods, autoclaving and boiling denatured; pasteurization was unreliable; freezing and thawing reduced numbers but did not sterilize; centrifugation at 100,000 $\times$ G for 1 hour with and without added gelatin did not produce a sterile supernate; prefiltration through glass wool adsorbed enzymes; filtration through Seitz or membrane filter adsorbed enzymes, filtration through fritted glass was impractically slow; sonic oscillation at 9 kc for 1 hour did not sterilize; gamma irradiation with 5×10^6 r from a Co^{60} source sterilized liquid saliva with protein destruction and was unreliable in the treatment of lyophilized saliva; irradiation with ultraviolet sterilized 73 per cent of the samples and usually did not reduce enzyme concentrations below the normal ranges. Thus the first- and the last-named technics were the most acceptable.

IV. WOUND HEALING AND OTHER INVESTIGATIONS. MARCH 17, AFTERNOON, SECTION D

37. EFFECT OF DIPHENYLHYDANTOIN SODIUM AND ANALOGUE ON WOUND HEALING.—*E. E. Kelln and R. J. Gorlin, University of Minnesota, School of Dentistry, Minneapolis.* One hundred and twenty Sprague-Dawley rats, 6-week-old males, were divided equally into four groups. In each group, half received the drug and half a diluent over an 18-day period. On the fifteenth day, abdominal wounds were made and were tested for tensile strength on the eighteenth day. All wounds were sutured, and only those healing by primary intention were used. Group I received 50 mg. of diphenylhydantoin sodium* per day by tube feeding; Groups II and III received 25 mg. of diphenylhydantoin sodium per day by intramuscular injection. Group IV received

* Dilantin Sodium Kapseals, Parke, Davis and Co.

15 mg. of 3-ethyl-5-phenylhydantoin* per day intramuscularly. A special instrument was designed for testing the tensile strength of individual wounds. Tensile strength was measured as a function of the number of milliliters of bromoform required to rupture the experimental wound. When statistically analyzed, diphenylhydantoin sodium and 3-ethyl-5-phenylhydantoin enhanced the tensile strength of wounds in all series.

38. Histogenesis of Healing of a Split-Thickness Flap in Dogs.—*Harry Staffileno, Balint Orban, and Frank Wentz, Department of Periodontics, Loyola University School of Dentistry, Chicago.* Clinical observation of healing of mucoperiosteal flaps in oral surgical procedures has indicated more or less complete regeneration of the part. The histogenesis of the healing process, the reaction of connective tissue, epithelium, bone tissue, and cementum of the surgical intervention were not studied in detail. This investigation was therefore undertaken to study the histogenesis of healing of the dentogingival junction after a split-thickness gingival flap was performed. The experimental subjects were four adult dogs with completely erupted permanent teeth. The surgery was executed in a manner simulating that performed in humans in the region of the premolar teeth. Animals were sacrificed at 0 hours and at 2, 6, 14, 21, and 60 days after operation. The findings were as follows: (1) The initial and predominant action was that of the epithelium. In 6 days the wound was completely sealed. (2) The connective tissue response was concerned with the initial mobilization of osteoclasts in 2 days, followed by bone resorption, then final bone regeneration. This reaction of the connective tissue extended over a period of from 2 to 21 days.

39. (Not presented.)

40. Histogenesis of Plasma Cells in Inflamed Oral Mucosa.—*Patrick D. Toto and Howard Bollinger, Loyola University, Chicago.* The source of plasma cells in inflamed oral mucosa has not been adequately studied. Our objective was to examine inflamed oral mucosa to determine, if possible, the histogenesis of plasma cells. Specimens of chronic inflammatory hyperplasia of the gingiva, lining mucosa of the vestibule, and edentulous ridges served as sources of material. The tissues were fixed in formalin or were fresh-frozen. Sections were cut at 6–10 μ and stained by hematoxylin and eosin, P.A.S. (Coleman) for mucopolysaccharides and for RNA activity. The frozen sections were treated with antihuman gamma globulin rabbit serum bound to fluorescent isothiocyanate. Plasma cells were invariably found immediately around the capillary. The accumulation of plasma cells varied from very few adjacent to the capillaries to diffuse infiltration extending into the collagenous fibers to fuse with another perivascular focus of plasma cells. The undifferentiated mesenchymal cells showed RNA activity in the cytoplasm. Immature plasma cells—proplasmocytes—showed increased RNA activity and centrally placed nuclei. More peripheral to the capillaries, the plasma cells matured, and showed an increase in cytoplasm mass with globule formation. The nucleus was pushed over to an eccentric position in the cytoplasm. Usually a pale halo was found between the nucleus and the cytoplasm protein. The cytoplasm did not stain with P.A.S., indicating the absence of mucopolysaccharides. Frozen sections treated with antihuman gamma globulin bound to fluoroscene isothiocyanate showed fluorescence of plasma-cell foci. This indicated the presence of gamma globulin in the plasma cells. Plasma cells appeared to arise from undifferentiated mesenchymal cells around the capillaries. They showed immature "proplasmacytes" with centrally placed nuclei and a small amount of cytoplasm. The RNA activity was increased as the cells' cytoplasm protein increased and showed Russell bodies. With increase in cytoplasm protein, the nucleus shifted to an eccentric position, assuming the morphology of the mature plasma cell.

* Peganone, Abbott Laboratories.

41. Pathogenesis of Mucocele or "Retention Cyst."—*Don H. Reynolds and A. P. Chaudhry, School of Dentistry, University of Minnesota, Minneapolis.* This study included clinical and experimental investigation into the pathogenesis of mucocele. The clinical part was based on the history and gross and microscopic features of human mucoceles. The experimental work dealt with the production of mucocele-like lesions in rats. Three groups of male white rats, 4 months of age, were distributed so that each group contained a equal number of animals of comparable weights. Under nembutal and ether anesthesia, the right submanidbular gland in each animal was exposed through a ventral mid-line incision of the neck, and the duct was separated from surrounding fascia. In the first group, the duct was ligated; in the second, cut; and in the third, compressed with a hemostat. Half the animals from each group were sacrificed at 3- and 7-day intervals, respectively. The glands were removed, fixed in 10 per cent formalin, and stained with different stains. The results of these clinical and experimental studies substantiate the findings of Bhaskar and his co-workers. The formation of mucoceles was due to the escape of mucus into the lamina propria and submucosa after trauma to the excretory duct. There was no evidence to support the commonly held hypothesis that mucoceles are caused by obstructions in the excretory ducts of minor salivary glands. This was further supported by the fact that in none of the cases of sialolithiasis of the minor salivary glands seen in our laboratory were there gross or microscopic attributes of a mucocele. This study, however, did not rule out the possibility that in occasional cases partial obstruction could have been responsible for dilatation and formation of the so-called "retention cyst."

42. Effects of Aging on Exteriorized Rodent Oral Mucous Membranes.—*Jules Klingsberg, Louis Cancellaro, and Earl O. Butcher, Department of Anatomy, College of Dentistry and Graduate School of Arts and Science, New York University.* This study was concerned with the effects of aging on the adaptive capacity of the oral mucous membrane of Syrian hamsters. The environment of the mucous membrane of 45 animals ranging in age from 1 month to 2 years was altered by two methods. The first method involved the extrusion of the cheek pouch through the skin of the neck, placing the mucous membrane surface of the pouch in direct contact with the external environment, and maintaining it in this position. The second method involved exposing the labial gingiva of the mandibular incisors to the atmosphere by surgically removing a portion of the lower lip. The pouches were examined histologically after 17 days of exposure, and the gingiva after 6 weeks. The stratum corneum of the control pouches of 1-month-6-month, and 2-year-old animals measured 7–10 μ in thickness. The corneum of experimental pouches of the 1-month- and 6-month-old animals was found to have undergone a threefold increase in thickness. Examination of the experimental pouch of the 2-year-old hamsters revealed a twofold increase. In the same manner, measurements were made of the control and experimental gingiva. The results indicated an increase in keratinization in the 1-month- and 6-month-old animals, which substantially exceeded the increase found in the 2-year-old animals.

43. Transfer of Certain Blood Constituents via Capillaries in the Pulp.—*John Haldi, Winfrey Wynn, and W. D. Culpepper, Emory University School of Dentistry, Atlanta, Georgia.* The presence of a capillary network in the dental pulp would appear to indicate that there may be a transfer of certain blood constituents through the capillary wall, as occurs in soft tissues. Radioactive materials such as P_{32} can pass through the capillaries, as shown by deposition in the tooth substance after parenteral injection and protection against entrance into the tooth from the saliva. When a small hole is drilled carefully with an Airdent, the pulp chamber can be reached without damaging the capillaries. A small glass or plastic tube can then be inserted in the hole and sealed in place. A colorless fluid rises slowly in the tube, and after several hours a sufficient quantity can be obtained for chemical analysis. Microscopic examination of a smear of this fluid revealed the absence of erythrocytes, which was taken to indicate that the capillary walls were not injured. Venous blood samples were drawn

simultaneously with the collection of the dental-pulp fluid. Blood plasma was analyzed for nitrogen, and whole blood for glucose. The same analyses were made on the dental-pulp fluid. The average protein content ($N \times 6.25$) of the blood plasma was 6.6 gm. per cent; that of the dental pulp fluid, 1.2 gm. per cent. Glucose, unlike proteins of the blood, passes freely through the capillary wall. The glucose content of the dental-pulp fluid was higher than in the blood, presumably because it reflected the concentration in the arterial blood. Investigations are under way to settle this question.

44. (Not presented.)

45. Exposure Time and Visibility of Steps of an Aluminum Penetrometer.—*H. D. Spangenberg, Jr., and M. L. Pool, Ohio State University, Columbus.* If the blackening under the various steps of an aluminum step-wedge penetrometer is plotted against the thickness of the aluminum step, a series of curves is produced. The greater the exposure time, the greater will be the roentgenographic blackening. For one example, if the roentgenograms are exposed to an unfiltered 70-kv X-ray beam, the density difference between the 1.5-cm. step is 0.05 if the background density is 2.00. If the density difference for the same aluminum steps are measured when the 0.1-cm. step yields a density of 2.00, the density difference is 0.09; when the 0.2-cm. step yields a density of 2.00, the density difference is 0.15; when the 0.4-cm. step yields a density of 2.00, the density difference is 0.25. For another example, if the roentgenograms are exposed to a 90-kv X-ray beam filtered by 0.375 mm. of copper and 0.5 mm. of aluminum, the density difference of the same aluminum steps is 0.15 if the background density is equal to 2.00; 0.25 if the 0.1-cm. aluminum step is equal to a density of 2.00; 0.27 if the 0.2-cm. aluminum step is equal to a density of 2.00; and 0.33 if the 0.4-cm. aluminum step is equal to a density of 2.00. In addition, similar data were obtained with X-ray radiations produced by other voltages and filter combinations. Radiations from radioactive sources were also used. From all the data studied, it was concluded that the visibility of the aluminum steps of an aluminum step-wedge penetrometer actually increases with the increase in exposure time.

46. Periodontal Changes Following Multiple Sublethal Doses of X-Irradiation in the Mouse.—*Max Shapiro, Ved Brat, and Benjamin H. Ershoff, Dental Research Division, Western Biological Laboratories, Culver City, California.* Male mice of the Webster strain were selected at weaning and were fed ad libitum a highly purified diet complete in all known nutrients. After 6 weeks of feeding, the animals were divided into two groups. One group received 6 weekly exposures of 200 r total-body X-irradiation, amounting to a total dose of 1,200 r. The second group served as non-irradiated controls. One hundred days after the last X-irradiation, surviving mice in both groups were sacrificed, and sections of the head were prepared for microscopic study. Periodontal changes were observed in all radiated animals, characterized by severe alveolar bone loss and periodontal pocket formation involving both the interradicular and the interdental areas. The epithelium had migrated apically to form extensive pockets with ulceration. Extensive deposits of amorphous material were present in the pocket area. A dense inflammatory exudate was noted in the connective tissue. The microscopic appearance of the pathologic areas closely resembled that of periodontal pockets in man. The above findings were not observed in non-irradiated controls. Caries did not occur in either group.

47. A Method of Measuring the Frequency of Swallowing in Man.—*Alexander C. Kerr, Clement S. Lear, and Sheila J. Moody, Forsyth Dental Infirmary, Boston.* The development of this method was prompted by speculation about a possible relationship between salivary flow rate and swallowing frequency, particularly during sleep. Observations with a stethoscope showed that the act of swallowing was always associated with a short, sharp, distinctive sound, best heard between the anterior border of the sternomastoid muscle and the thyroid cartilage. A miniature microphone mounted in a plastic box was held over this position by an elastic neckband. Signals

from the microphone were conducted to a tape recorder. A repeated 5-minute time signal was concurrently recorded on the same tape. The recording speed generally used was $1\frac{7}{8}$ i.p.s. At playback, swallowing sounds could be clearly identified at tape speeds of $1\frac{7}{8}$ and $3\frac{3}{4}$ i.p.s. For long records the tape was scanned at four times the recording speed, and the identity of interesting sounds was confirmed by replaying at $1\frac{7}{8}$ i.p.s. Recordings were made from adults who were sitting, eating, reading, writing, watching television, or lying supine, awake or asleep. The subjects' swallowing rates were not affected by consciousness of the experimental situation. Sedentary individuals swallowed between 20 and 40 times per hour. Lying supine reduced the rate by about 50 per cent. During sleep, swallowing was infrequent. In one subject 9 recordings of the first 4 hours of sleep gave total counts of swallows ranging from 8 to 28. The higher counts were associated with restlessness and coughing during sleep. The frequency of swallowing rose as high as 9 per minute during eating.

48. AN ELECTRONIC METHOD FOR MEASURING EXTRAORAL CERVICAL TRACTION FORCES.—*T. J. Zwemer and A. B. Chase, College of Medical Evangelists, Loma Linda, California.* The efficacy of various centric and eccentric cervical traction appliances was studied by measuring electronically the forces delivered to the point of molar attachment. 120-ohm strain gauges* were mounted on either side to two stainless-steel spatulas, to which had been soldered 0.045 stainless-steel tubes. These strain gauges were connected to a strain-gauge analyzer and recorder.† The two strain gauges were calibrated against known analytical balance weights through a range of 50 gm. Five facebow designs were constructed—a bilaterally symmetrical extraoral bow mounted rigidly at the mid-point of a symmetrical intraoral arch; an asymmetrical extraoral bow rigidly fixed eccentrically to a symmetrical intraoral arch; and an extraoral bow with unequal arms mounted rigidly to the mid-point of a symmetrical intraoral arch. In addition to these rigid types, two specially constructed hinged bows were tested and compared with the above-mentioned rigid bows—a bilaterally symmetrical extraoral bow hinged at the mid-point of a symmetrical intraoral arch and an asymmetrical extraoral bow hinged eccentrically to a symmetrical intraoral arch. Each of the above facebow designs was tested by three types of loading—the application of equal weights to the lateral terminals of each extraoral bow, the application of unequal weights to the lateral terminals of each extraoral bow, and the application of elastic traction via the use of a Kloen-type cervical strap. Both hinged and rigid bilaterally symmetrical appliances responded identically to equal loading. When unequally loaded, the behavior was quite different. The hinged bow continued to deliver equal force to each molar attachment, whereas the rigid bow delivered a greater force to the molar on the side of greater loading. The three unilateral appliances tested all delivered a greater force to the site of one molar attachment than to the other. The magnitude of this difference varied considerably with each appliance. The hinge bow delivered 73–26 gm., the eccentric bow delivered 53–47 gm., and the unequal arms bow delivered 60–40 gm. when loaded with 100 gm. equally divided between the two lateral terminals. The simple expedient of altering the connection between extraoral bow and intraoral arch can markedly change the character of force delivered to the site of molar attachment.

V. FLUORIDES AND VITAMIN-D DEFICIENCY. MARCH 18, FORENOON, SECTION A

49. TIN AND FLUORIDE REACTIONS WITH ENAMEL: CONCENTRATION AND pH EFFECTS.—*William E. Cooley, Miami Valley Laboratories, Procter & Gamble Co., Cincinnati.* Sound, pre-etched pieces of incisor enamel were continuously brushed while immersed in the treating solution. After treatment, the tin and fluoride in the enamel were determined in perchloric acid solutions of the treated samples. Measurable amounts of tin were deposited on enamel by very dilute tin fluoride solutions (0.00001

* Baldwin S-R-4, type A-5-1.

† Sanborn Twin-viso.

M), and the amount deposited increased with concentration until it was five times as much for a given reaction time at 0.001 M (Sn, 120 ppm) as at 0.00001 M (Sn, 1.2 ppm). With further increase up to 0.1 M, there was little change in reaction rate, so that all solutions in this middle concentration range were equally effective in laying down tin on the enamel. Above 0.1 M the process was mainly adhesion of precipitate, and large quantities of tin accumulated on the enamel surface. Solutions whose pH was higher than natural also reacted by the adhesion process. Dilute sodium fluoride solutions provided fluoride to the enamel at a rate that increased with concentration between 0.001 and 0.05 M, remained nearly constant up to 0.2 M, then increased again up to saturation (1 M). At higher fluoride concentrations, tin fluoride solutions supplied fluoride to enamel more rapidly than did sodium fluoride solutions. The rate difference was especially great above 0.1 M, because fluoride was partly occluded in adhering precipitate from tin fluoride solutions.

50. Two-Year Results of Single SnF_2 Application and a Stannous Fluoride-containing Dentifrice.—*Joseph C. Muhler, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* It has previously been reported (*J. D. Res.*, **38**:994, 1959) that children using a stannous fluoride dentifrice in an unsupervised manner had approximately 30 per cent less tooth decay when the DMFT index was used and 20 per cent less when the DMFS index was used to evaluate dental caries experience than a comparable control group at the end of 1 year. When the group received a combination of topical stannous fluoride and a stannous fluoride dentifrice, approximately 60 per cent less new caries was found by either the DMFT or the DMFS index. This report concerns the findings at the end of 18 and 24 months. At the end of 18 months, 200 control children using a stannous fluoride dentifrice had a DMFT and DMFS increment of 2.04 and 4.72, respectively; 211 children had an increment of 1.30 and a 3.43 DMFT and DMFS, respectively; and 215 children who received a single topical application of an 8 per cent solution of stannous fluoride every 6 months and, in addition, used a stannous fluoride dentifrice had a DMFT and DMFS increment of 0.84 and 2.08, respectively. At the end of 24 months the control group (193 children) had a caries increment of 2.49 and 5.91, respectively, for DMFT or DMFS. The group using only the stannous fluoride dentifrice had an increment of 1.66 (189 children) and 4.41, respectively, when the DMFT or DMFS index was used, while the group receiving both the topical and the dentifrice (199 children) had an increment of 1.04 for DMFT and 2.64 for DMFS. These results confirm the findings at the end of 1 year and show that a combination of topical stannous fluoride and the routine use of a stannous fluoride dentifrice is an effective anticariogenic procedure.

51. Single Application of Stannous Fluoride Results after Five Years.—*Charles W. Gish, Joseph C. Muhler, and Charles L. Howell, Indiana State Board of Health and Indiana University, Indianapolis.* The clinical results obtained at the end of 1, 2, 3, and 4 years following the use of a single application of an 8 per cent aqueous solution of stannous fluoride compared with a series of four applications of a 2 per cent solution of sodium fluoride showed an anticariogenic superiority of the single application of stannous fluoride by about 30 per cent when evaluated by either the DMFT or the DMFS index. This report summarizes the data obtained at the end of 5 years and shows that the group receiving five single applications of stannous fluoride 1 year apart had a total caries increment of 2.04 DMFT and 2.92 DMFS, while the comparable group receiving sodium fluoride had a total increment of 3.31 DMFT and 5.25 DMFS. The groups beginning 4 years ago and receiving four single applications of stannous fluoride had a total caries increment of 1.47 DMFT and 2.81 DMFS, while the comparable group receiving sodium fluoride had a total increment of 2.30 DMFT and 4.03 DMFS. (The expected increment for this area is 5.10 DMFT for a 5-year period.) These data demonstrate the superiority of a single application of stannous fluoride over the four-application technique using sodium fluoride.

52. (Not presented.)

53. CLINICAL EVALUATION OF PATIENTS' TASTE ACCEPTANCE OF A STANNOUS FLUORIDE, SILEX, SILICONE, DENTAL PROPHYLAXIS PASTE.—*V. A. Segreto, R. Detamore, L. Lightner, S. Benhart, N. O. Harris, and W. R. Hester, School of Aviation Medicine, USAF, Brooks Air Force Base, Texas.* A stable stannous fluoride, silex, silicone, dental prophylaxis paste with potential anticariogenic properties, developed at the School of Aviation Medicine, was clinically tested at four U.S. Air Force bases to determine patients' taste acceptance, the paste's polishing capabilities, and possible contraindications. Four hundred and twelve cases were evaluated. Using a scale with a range of 0–10 points (good = 10, bad = 0), patients' estimation of taste averaged 4.5, hygienists' evaluation of patients' reaction averaged 5.4, and hygienists' evaluation of polishing capabilities averaged 5.1. All male military patients, except one, indicated that therapeutic potentialities outweighed any taste consideration. Mild papillary and marginal blanching of the gingiva occurred in the majority of the cases. This disappeared within a few days without treatment or discomfort to the patient. In questionable anterior restorations a few cases of staining of the marginal areas occurred. On the basis of these data it was concluded that taste is the major contraindication to routine clinical application.

54. EFFECT OF MOLYBDENUM ON FLUORIDE RETENTION IN THE RAT.—*George K. Stookey and Joseph C. Muhler, Indiana University Medical Center, Department of Biochemistry, Indianapolis.* Previous experience in this laboratory has suggested that the presence of molybdenum is associated with an increased skeletal retention of fluoride. In order to investigate the mechanism by which this increased skeletal retention occurs, experiments were performed to evaluate the metabolism of fluoride in the presence or absence of molybdenum. The amount of fluoride in various soft tissues was also studied. The results demonstrated that the presence of molybdenum increased skeletal fluoride retention, and the metabolism data corroborated this by demonstrating less fluoride excretion in the group receiving molybdenum. Elevated levels of fluoride in the soft tissues also appeared to be related to the presence of molybdenum.

55. THE GASTRIC ABSORPTION OF FLUORIDE.—*Martin J. Wagner, Baylor University College of Dentistry, Dallas, Texas.* Several workers have suggested that fluoride is absorbed rapidly from the gastrointestinal tract. This study was concerned with determination of the rate of absorption of aqueous fluoride by the gastric mucosa. Approximately 100 Sprague-Dawley rats were used. Following 6–12 hours' fasting, each animal was placed under sodium pentobarbital anesthesia. The pyloric valve was exposed, with care taken to minimize trauma to the surrounding tissues and blood supply. The valve was ligated, and, following ligation, 0.5–1.0 ml. of a 30 ppm F (as NaF) solution was injected into the lumen of the stomach. The incision was then closed with silk suture or small skin clips. After intervals ranging from 5 minutes to 5 hours, the cardiac valve was ligated and the stomach excised, and the time elapsing between injection and excision was noted. After ashing, the sample was analyzed for the residual fluoride, using a perchloric acid distillation and a thorium nitrate titration. Control animals were injected similarly with 1 ml. of isotonic NaCl solution, in order to nullify the contribution of any tissue fluoride. In addition, the injected quantity of fluoride was analyzed separately, in order to establish a reference point for calculation of recovery percentages. The results of these analyses showed that the gastric mucosa was capable of absorbing the fluoride ion efficiently. The amount of fluoride remaining in the stomach decreased nearly exponentially with elapsed time.

56. FLUORIDE AND CALCIUM CONCENTRATIONS IN HUMAN DENTAL PULPS.—*Shin H. Yoon, Finn Brudevold, Frank A. Smith, and Dwight E. Gardner, Forsyth Dental Infirmary and the Harvard School of Dental Medicine, Boston, and University of Rochester, Rochester, New York.* F and Ca were determined on pooled pulps of teeth from various age groups (under twenty, twenty to twenty-nine, thirty to forty-nine, and over fifty) from communities with 0.06, 1.0, 3.0, and 5.2 ppm of F in the water supply. Almost without exception, there was a marked increase in the concen-

tration of F with age. For example, in the pulps from Big Lake, Texas, the concentrations of F on a dry-weight basis were 358, 1,033, and 1,541 ppm for the under-twenty, twenty to twenty-nine, and over-fifty-year groups, respectively. The increased deposition of F appeared to be associated with a similar increase in the concentration of Ca. Pulps from areas with high levels of F in the water generally had higher concentrations of F than did those from low-F areas. In contrast to Ca and F, which showed increased deposition with age, there was decreased water content in old compared with young pulps.

57. Formative Fluoride and Size of Cusp of Carabelli.—*Gerald J. Cox, University of Pittsburgh, Pittsburgh, Sidney B. Finn, University of Alabama, Birmingham, and David B. Ast, Department of Health, Albany, New York.* On the basis of the observation of absence of the cusp of Carabelli in the dentitions of six persons with frank mottled enamel and the recorded significant absence of the mesiobuccal cusp of the upper third molar teeth of rats born to mothers on a high fluoride ration, 764 casts of the dentitions of children of Newburgh and Kingston, New York, were examined for the size of the cusp of Carabelli. Of 669 casts with the first molar teeth present, 318 were from Kingston and 351 from Newburgh. The identity of the source of the casts was not known to the examiner. The casts were first classified into eight groups as to size of the cusp and were then brought into two groups. In the Kingston casts, 159 were in each group; in the Newburgh casts, 153 were in the smaller sizes, or missing, and 198 were in the larger sizes. The chi-square test of a 2×2 table of these values showed a probability of 0.1 that larger cusps of Carabelli would be produced by formative fluoride in drinking water under the climatic conditions of these cities.

58. Effect of Low Doses of Sodium Fluoride on Tooth Development.—*J. C. Parikh, Georgetown University, School of Medicine and Dentistry, Washington, D.C.* The purpose of this experiment was to determine the minimal concentration of sodium fluoride capable of producing microscopic disturbances in developing teeth of rats. Seven litters of newborn rats were used in this study. Beginning on the third day postnatal, animals of five litters were administered sodium fluoride daily by intraperitoneal injections in doses ranging from 6 to 50 μg. F. The sixth group was given an equal amount of distilled water to determine the effect caused by the stress of injection. The seventh group did not receive any treatment. The animals were sacrificed after 2, 3, 5, 7, 10, 12, 15, and 18 doses. The control animals were sacrificed at the same intervals. Jaws containing developing teeth were fixed in Bouin's solution for 24 hours, decalcified by Morse's technic, cleared in alcohol, and imbedded in paraffin. Serial sections were cut at 6 μ thickness and stained with hematoxylin and eosin and Masson trichrome for microscopic examination. The observations indicated that only animals receiving 50 μg. F showed changes in the developing teeth. The effect on the ameloblastic layer and retardation in the calcification of enamel matrix were evident. The dentin showed an interglobular texture. Mild hyperemia of the pulp was sometimes seen. The teeth of animals in all other groups failed to reveal any disturbances, suggesting that the young rats were able to tolerate these amounts of sodium fluoride.

59. Effect of Vitamin D Deficiency on Tooth Development.—*Enrique Kiguel, Georgetown University, School of Medicine and Dentistry, Washington, D.C.* The role of vitamin D in calcification of enamel and dentin has been generally recognized. A deficiency of this vitamin during tooth formation has been reported to cause alterations in composition and structure. This investigation was therefore designed to determine the influence of vitamin D deficiency in the developing teeth of two groups of rats. The severity of deficiency in animals fed Steenbock diet (Ca:P ratio = 4–5:1) produced rickets, whereas the other group received a vitamin D–free, non-rachitogenic diet (Chick Basal diet, Ca:P ratio = 1–2:1). A total of 24 adult female albino rats (two groups of 12 animals each) were placed on the above-mentioned diets for 3 weeks to deplete all their vitamin D reserves. Mating of these rats was started. The offspring

from these female rats (37 animals from the Steenbock group and 16 from the Chick Basal group) were used in this experiment. Animals receiving nutritionally adequate diets were used as controls. All animals were sacrificed at 3-day intervals beginning from birth to 21 days postnatal. Serial sections were made of the jaws from each animal for histologic examination. The observations suggested that almost all animals in both groups showed abnormalities in amelogenesis and dentinogenesis to a varying degree. The enamel matrix was fragile and of a poor texture. The odontoblastic layer appeared to be severely affected. Capillary invasion and a loss of cellular detail were evident. Disturbances in the calcification of dentin matrix was a common feature. In general, the changes in the teeth of the Steenbock group were more severe than in the Chick Basal group.

60. Effects of Fluoride on Tooth Development of Vitamin D–deficient Animals.—*B. R. Bhussry, Georgetown University, School of Medicine and Dentistry, Washington, D.C.* The purpose of the present study was to determine the influence of various doses of sodium fluoride upon the calcification of teeth in animals fed a vitamin D–deficient diet. The animals used in this study were from the litters born of female rats fed on the Steenbock diet (Ca:P ratio = 4–5:1) and Chick Basal diet (Ca:P ratio = 1–2:1). Both these diets produce a vitamin D deficiency. Beginning at 3 days of age, the newborn young rats received sodium fluoride subcutaneously in amounts ranging from 25 to 113 μg. F. The experimental animals were sacrificed after administration of 2–15 doses of sodium fluoride. Animals receiving the vitamin D–deficient diets and nutritionally adequate diets were used as controls. Serial sections of the jaws from each animal were prepared for histologic examination. Comparisons were made on animals of similar ages in the control and experimental groups. The influence of sodium fluoride on the developing teeth was apparent in both groups of animals receiving vitamin D–deficient diet. The effect was dependent on the amount of fluoride administered and the severity of vitamin D deficiency. The developing teeth showed hyperemia of the stellate reticulum, compression of the ameloblasts, and a disturbance in the intercellular substance of the ameloblastic layer. Capillary invasion of the odontoblastic layer was obvious. The calcification of dentin matrix was retarded, and interglobular dentin was formed. Whether the influence of vitamin D deficiency and sodium fluoride on tooth development is synergistic or antagonistic is still open to speculation.

VI. ANATOMY AND PHYSIOLOGY. MARCH 18, FORENOON, SECTION B

61. Factors of Tooth Rotation and Axial Inclination of Lower Premolars.—*Y. H. Kim, H. R. Fogels, and F. R. Shiere, Tufts University School of Dental Medicine, Boston.* Deviations from the norm in tooth rotation and in axial inclination of erupting lower premolars were studied. Four different conditions were chosen: normal pattern (Group I), asymmetrical root resorption of deciduous molars (Group II), pathologic involvement in the apical region of deciduous molar roots (Group III), and early loss of the first permanent molar prior to the eruption of adjacent premolars (Group IV). Measurements taken showed that Group I had less than three degrees of mean rotation in erupting lower premolars. Groups II, III, and IV had considerable rotation and were found to be significantly different from Group I at the 1 per cent level ($P < 0.01$) in each group analyzed. In the first three groups, the axial inclination in relation to the occlusal plane was found to be 88 degrees (a slight distal inclination) for the lower first premolars and 92 degrees (a slight mesial inclination) for the second premolars. These teeth showed a marked distal inclination in Group IV. The mean difference of axial inclination between Groups I and IV was found to be significant at the 1 per cent level ($P < 0.01$) in both the lower first and second premolars. From these findings the following conclusions were drawn. The preservation of a badly infected deciduous molar for the purpose of preventing space loss is contraindicated. Extraction of lower first permanent molars prior to the eruption of adjacent premolars should be avoided.

62. Spacing in the Deciduous Arch.—*James E. McIver, State University of Iowa, Iowa City.* Characteristics of the spacing or crowding in the mandibular deciduous arch of 46 Caucasian females were studied. The characteristics included central tendencies of the spacing, change in spacing during the period of the deciduous dentition, and relation of the changes in spacing to change in the anterior arch width. The spacing or crowding was measured on casts from the Facial Growth Study conducted at the State University of Iowa. The spacing or crowding between the deciduous teeth anterior to the second deciduous molars was measured on the consecutive casts at 4 years and at each subsequent 6-month interval, as long as the deciduous arch remained intact. Spacing was measured with round wires of known diameters, and crowding was ascertained by measuring the individual teeth involved and subtracting the arch space occupied. Maximum and minimum arch widths at the canines were taken at each age. Correlations were obtained between (*a*) the total spacing at 4 years and the total spacing at the last age of the intact deciduous arch; (*b*) the individual space areas at 4 years and those same spaces at the last age that the deciduous arch was still intact (7 correlations); and (*c*) the change in spacing from 4 years to the last age of the intact deciduous dentition and the change in the maximum and minimum canine arch width between the same ages. Correlations for *a* and *b* were between $r = 0.9$ and 0.8 and for *c* between $r = 0.6$ and 0.3. The average arch spacing at 4 years was 3.1 mm., and at the last age of the complete deciduous arch, 2.9 mm.

63. Comparative Relationships of Tooth Material and Supporting Bone.—*Leonard A. Altemus, Howard University College of Dentistry, Washington, D.C.* The purpose of this study was to compare the relationship of tooth material to its supporting bone in the dentitions of two segments of the population of the United States. The dental models of 80 Negro children, 40 boys and 40 girls, with normal tooth occlusion (angle) were measured according to the methods of Ashley Howes. These children were of Hellman's dental age IV A, with complete permanent dentitions except for the third molars. Dentitions requiring no orthodontic treatment were considered as having normal occlusion. The measurements were made at the College of Dentistry, Howard University, Washington, D.C.

The means of the above measurements were compared with those of Ashley Howes for Caucasian children. The findings are given in the table.

	Tooth Material (TM)	1st Bicuspid Coronal Arch Width (BCAW)	BCAW/TM	1st Bicuspid Basal Arch Width (BBAW)	BBAW/TM	Basal Arch Length (BAL)	BAL/TM
Maxillary arch:							
Howes	91.7	41.6	45.5	43.8	47.7	32.4	35.4
Howard	106.1	44.1	41.6	46.8	44.2	40.0	37.9
Mandibular arch:							
Howes	84.1	33.9	40.5	39.9	47.1	31.4	37.1
Howard	97.6	36.2	37.1	40.5	41.5	38.3	39.2

A comparison of these data shows that, in the Howard group, tooth material was larger, the coronal and basal arch widths were wider, and the basal arch lengths were longer. In this group also the percentage relationships of the tooth material to the coronal and basal arch widths were smaller, whereas the percentage relationships of the basal arch lengths to the tooth material were greater.

64. Roentgenographic and Histologic Differentiation of Dentinogenesis Imperfecta and Dentinal Dysplasia.—*William V. Bernard, University of California, School of Dentistry, San Francisco.* An investigation was undertaken to understand better the dental anomalies of dentinogenesis imperfecta and dentinal dysplasia. It

appeared that these two anomalies have been described by many as the same condition. Three genetic pedigrees were obtained, two of which covered five generations. Ground and decalcified sections prepared from primary and permanent teeth showed occluding pulp chambers; however, the dentinal tubules of dentinal dysplasia ran a more tortuous course, at times in a horizontal direction, being interrupted by imbedded pulp stones. The following conclusions were drawn from this investigation: (1) dentinogenesis imperfecta is a dental anomaly affecting both primary and permanent dentitions genetically exhibiting a single dominant characteristic, non-sex-linked; (2) dentinal dysplasia was found to have a hereditary association in one family, in contrast to previous reports; (3) both these anomalies usually have an occluded pulp chamber; (4) dentinogenesis imperfecta has typical "bell-shaped" crowns with foreshortened narrow roots; (5) the shape of the root in dentinal dysplasia is much different, varying from practically no root at all to a very elongated root (many of the molars present a "w-shaped" appearance); (6) dentinal dysplasia has a tendency for the roots to form periapical rarefactions, which is not true with dentinogenesis imperfecta; (7) the enamel in cases of dentinogenesis imperfecta is probably always affected in degrees from slight to severe (these teeth are not caries-immune); (8) there is as yet insufficient proof to associate definitely the dental anomaly which often occurs in osteogenesis imperfecta with dentinogenesis imperfecta.

65. Enamel Thickness in Pits and Fissures of Human Posterior Teeth.—*B. Gillings and M. Buonocore, Eastman Dental Dispensary, Rochester, New York.* Pooled non-carious molars and bicuspids were used to investigate enamel thickness at the base of pits and fissures in occlusal surfaces. Fifty teeth were mounted in square plastic blocks and sectioned buccolingually at 120-μ thickness, at 450-μ intervals, using a sectioning machine. With the square edges of the plastic support as reference coordinates, 25$\times$ tracings were made on graph paper by means of a projecting microscope. These tracings made possible (1) the measurement of radial enamel and dentin thickness at any point; (2) the measurement of pit and fissure depth; (3) the visualization of other planes of section and the spatial configuration of the pit and fissure areas; and (4) the construction of large three-dimensional models. In practically every tooth sectioned, some areas of the fissures had little enamel over the dentin. It was found that fissures frequently constricted close to the opening on the tooth surface. Attempts, therefore, to determine fissure depth in these areas by explorer will give misleading impressions. The deep fissure areas appeared to be filled with organic matter. Sections of unerupted teeth indicated that part of this matter was present before eruption. The suggestion is made that the almost universal presence of these deep fissure areas contributes to the frequency and rapid development of fissure caries for two reasons—their inaccessibility to cleaning and the greatly reduced enamel thickness at their bases.

66. The Dentition of the Kish Population, 3000 B.C.—*Virginia M. Carbonell, Dental Anthropology Laboratory, W. G. Zoller Dental Clinic, University of Chicago.* The morphology of the dentition of the Kish population, Mesopotamia, 3000 B.C., was studied and classified, in order to define any traits present specific to the group and to determine the evolutionary position of their development. The human materials reported in this study were excavated from Kish, Mesopotamia, which was situated between the Tigris and the Euphrates rivers. The cranial materials consisted of longheads and roundheads. The quantitative analysis of tooth size and shape was accomplished by measuring the mesiodistal length of each tooth with a metric gauge. The resultant data were subsequently submitted to statistical analysis and utilized to provide a description of the Kish dentition. The evolutionary development of the teeth of the Kish people closely resembled the dentition of the modern white population of the world today. The size of the Kish dentition presented reduction in size and simplicity in morphological structure compared with earlier known people of the world. Reduction in the size and number of cusps was observed in the molar teeth. Genetic

and evolutionary traits, such as dental tubercle and Carabelli's cusp, were observed. Shovel shaping was absent on the incisor teeth. Attrition, dental caries, and periodontoclasia affected the dental system of the Kish population. In general, the dentition of the Kish population presented advanced evolutionary characteristics closely resembling the dentition of white populations today.

67. Structural Features of the Temporomandibular Joint Disk.—*Andrew D. Dixon, Departments of Anatomy, State University of Iowa, Iowa City, and the University of Manchester, England.* This study was primarily concerned with the innervation of the intra-articular disk, in an attempt to clarify its possible role in the etiology of temporomandibular joint pain. Histologic examination of human post mortem material (forty-five to sixty years) showed the disk to consist of two distinct parts: a dense avascular anterior part composed of intricately woven fibrous tissue, in which small groups of chondroid cells could be seen in older subjects, and a posterior part of more open texture. The posterior part of the disk possessed a considerable amount of elastic tissue in its upper part, some of which formed a well-defined band extending upward and backward from the posterior aspect of the fibrous portion of the disk to the squamo-tympanic fissure. This band may be important in the return of the disk into the glenoid fossa during jaw closure. Inferiorly the posterior part of the disk contained a number of large vascular channels. In this spongy tissue, bundles of myelinated and non-myelinated nerve fibers could be demonstrated by a modification of the Bielschowsky-Gros silver-impregnation technic. These bundles, frequently in close relationship to vascular spaces, could be traced as far as the posterior limit of the dense fibrous part of the disk, where they terminated freely. It appeared that the posterior part of the intra-articular disk was sufficiently well innervated to elicit painful responses to inflammatory changes or compression, such as may result from persistent overclosure of the jaws.

68. Nerve Endings in Gingival Tissue.—*Dan E. Tolman, R. K. Winkelmann, and Joseph A. Gibilisco, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.* The purpose of this study was to investigate the structure, number, distribution, and histochemical aspects of nerve endings in fresh human, free gingival tissue. Further knowledge concerning the histologic structure of nerves of the gingival tissue, in the light of newer laboratory technics, is revealing and necessary for broadening our understanding of oral sensations. Cholinesterase and silver nitrate staining methods were used in this study. The gingival tissue for this experiment was obtained at the time of an oral surgical procedure. One half of the tissue obtained was placed in a solution of sucrose ammonium hydroxide and 10 per cent formalin and the other half in a cold solution of isotonic saline and 10 per cent formalin for fixation. Following fixation, the tissue was sectioned at 50 μ and stained for observation. Of the 28 patients involved in this study, 18 were females and 10 were males. Their ages ranged from three to seventy-eight years. The gingival tissue of the children in this study exhibited more nerve endings than did that of the older adults. More nerve endings per millimeter of gingival surface were observed in the incisor and cuspid region than in any other area of the mouth. The organized nerve endings observed and the cholinesterase activity recorded were discussed in terms of previous work in this field.

69. Vascular and Nerve Supply to the Temperomandibular Joint of Rats.*—*Sol Bernick, Department of Anatomy, University of Southern California, Los Angeles.* Rats of varying ages were used to study the vascular and nerve supplies to the temperomandibular joint. The visualization of the vascular patterns to the temperomandibular joint was accomplished by the India ink gelatin perfusion method. After the perfusion period the heads were fixed in an alcohol–acetic acid formol solution. The specimens were then decalcified and prepared for nitrocellulose imbedding in the routine manner. The imbedded blocks of tissue were sectioned at 100–200 μ thick-

* This investigation was supported by Research Grant D-1033 from the N.I.D.R., U.S. Public Health Service.

nesses, and the cut sections were cleared by the Spalteholz method and mounted. For the demonstration of the nerve distribution to the temperomandibular joint, heads from rats of the same age groups as used above were prepared for sectioning in the same manner as described above. Blocks of tissue as well as cut sections were first treated with enzymatic hydrolysis (1 per cent pepsin), hydrated, and silver-impregnated by Pearson's silver gelatin method. In the young animals a rich vascular network was traced into the various components of the temperomandibular joint, and both medullated and non-medullated nerves were observed accompanying the blood vessels in their distribution. In addition, changes due to aging were noted.

70. Blood Flow and Oxygen Saturation in the Common Facial Vein.—*Jack G. Bishop, D. R. Redden, J. L. Matthews, and H. L. Dorman, Department of Physiology, Baylor University College of Dentistry, Dallas, Texas.* Mongrel dogs were anesthetized with sodium pentobarbital (33 mg/kg given intravenously). The blood of the animals was rendered incoagulable by administration of 5 mg/kg of heparin. Arterial blood pressure and heart rate were continuously monitored by the use of a Statham strain gauge, Brush amplifier, and recorder, from the femoral artery. A cannulating electromagnetic blood flowmeter continuously recorded the volume rate of blood flow in the common facial vein. Venous oxygen saturation was simultaneously measured by a recording oximeter. Mean arterial blood pressure remained constant throughout the experiments, usually above 120 mm Hg. Heart rate was also observed to remain constant at about 150 beats per minute. The blood-flow rate fluctuated greatly as it passed through the blood-flow meter which was inserted into the common facial vein. The maximum flows recorded were near 20 cc/min and coincided with the inspiratory phase of respiration. During expiration the flow rate usually decreased to below 10 cc/min. The venous oxygen saturation, however, tended to remain relatively constant at about 65 per cent saturation. Very small increases in oxygen saturation of the blood in this vessel were nevertheless discernible as each increase in the blood-flow rate occurred.

71. Effects of Excessive Occlusal Forces in the Gingival Blood Supply.—*D. Walter Cohen, Gerard Keller, Morris Feder, and Edward Livingston, Department of Periodontology, Graduate School of Medicine, University of Pennsylvania, Philadelphia.* Gold crowns were fabricated and inserted on the maxillary and mandibular right first permanent molars of mangabey monkeys. These restorations ended supragingivally to prevent irritation within the gingival sulcus. When the crowns were cemented in place, the right first molars were the only teeth to contact each other in the various occlusal positions. Animals were sacrificed at 30 hours, 3 days, 5 days, 2 weeks, 2 months, 4 months, 6 months, 12 months, and 18 months. At the time of sacrifice all animals were perfused with 1 liter of refined black India ink following clearing of the vessels with about 1,500 cc. of 0.85 per cent sodium chloride solution. The solutions were injected into the right carotid artery in 4 animals, and in 9 animals both carotids were perfused simultaneously, with return flow coming through the jugular veins. The ink was prepared by repeated vacuum filtration, centrifugation, and microscopic examination during various stages of the procedure. Particles in the final product were less than 1 μ in diameter. The gingival tissues and teeth were photographed and the jaws radiographed before sectioning. The specimens were fixed in 10 per cent formalin for 48 hours. In order to visualize the pathways of the terminal vessels, the material was serially sectioned on a microsaw which permits serial cutting of undecalcified material. The sections (100 μ—1 mm. in thickness) was dehydrated in alcohol and cleared by using a solution of equal parts methyl benzoate and benzyl benzoate. Occlusal traumatic forces were induced in the experimental animals, and while the the typical changes occurred in the attachment apparatus the gingival blood supply was not affected.

72. Implant Electrodes: Their Possibility in Dental Research.—*Eastwood G. Turlington, Reginald G. Bickford, and Joseph A. Gibilisco, Mayo Foundation and*

Mayo Clinic, Rochester, Minnesota. Many recent advances in neurophysiology have been accomplished with the aid of intracranial and intracellular recording electrodes. These methods have facilitated many in vivo studies of nerve function. The physiology of the trigeminal nerve has been investigated by this procedure also. Since most technics require radical dissection of the brain, long-term evaluation in one animal has been difficult to attain. Following a cranial trephine, nichrome-wire recording electrodes were successfully implanted into the gasserian ganglion. A stereotaxic instrument was utilized to position the electrodes. The extracranial portion of each electrode was soldered to a miniature socket, which was affixed to the skull with stainless-steel screws and self-curing denture material. An indifferent electrode composed of a stainless-steel disk with a copper wire was connected to one pin of the miniature socket. The circuit was completed by attaching the leads to the amplifiers. Stimulation to peripheral fibers of the fifth cranial nerve resulted in an action potential which was observed and photographed on the oscilloscope. The electrodes remained in position for more than 1 year and were functioning throughout this period. Subsequent dissection and histologic evaluation verified the position of the electrodes within the Gasserian ganglion. This technic has been used in the analysis of the blocking effect of local anesthetics on nerve conduction.

VII. E. H. HATTON AWARD COMPETITION I. MARCH 18, FORENOON, SECTION C

73. CUTTING STUDIES OF VARIOUS-SPEED HANDPIECES WITH APPROPRIATE INSTRUMENTATION.—*P. R. Kulp, L. Kurlansik, and R. E. Plowman, Dentists' Supply Company of New York, York, Pennsylvania.* High rotational speed is known to make more effective use of small-sized burs and stones. In this study various bur and diamond instruments which would be appropriate for external cuts, opening internal cavities, etc., and which would also be appropriate for each of the speed ranges considered, were compared. The advantages of high-speed handpieces—reduced operating pressure, vibration sensibility, and small tool accessibility—were retained, but slow-speed, high-torque handpieces, when used with large stones and high pressures, were found to be capable of as rapid, or faster, rates of material removal.

74. THE EFFECTS OF VARYING WATER-POWDER RATIOS ON CLASS II STONES.—*Philip F. Schoen, Loyola University School of Dentistry, Chicago.* Many accurate impressions are spoiled because of faulty manipulation of the gypsum products from which the model is poured. Accuracy in measuring the powder and liquid is of prime importance. This paper is concerned with the effects of variations in water-powder ratio on the linear setting expansion, compressive strength, and setting times of four different brands of Class II dental stone.

Three different mixes of each of these stones were made—a thin mix, a normal mix recommended by the manufacturer, and a heavy mix. The thin and heavy mixes were made in an effort to simulate what might be used in some dental offices if the proportions were not measured but were judged by consistency alone. However, in order to arrive accurately at the same thin and thick consistency for all four brands of stone, it was necessary to perform slump tests on all of them, thereby standardizing the consistency. After this was done, the various samples were tested for the degree of linear setting expansion and compressive strength. The initial and final sets were also noted. The data were obtained through the use of a trough-type linear setting expansion device, a hydraulic crushing machine, and Gillmore needles. The readings were recorded and the linear setting expansion graphed for each of these mixes over a 36-hour period.

75. ELECTRODE COMPARISON FOR ELECTROMYOGRAPHIC PROCEDURES.*—*Daniel L. Sutton, College of Dentistry, Ohio State University, Columbus.* The purpose of this study was to determine the effects of different electrodes on electromyographic record-

* This investigation was supported by Research Grant D-548 (C-2) from the N.I.D.R., U.S. Public Health Service.

ings. Two types of electrodes in general use for dental electromyography were tested: (1) monopolar surface electrodes and (2) monopolar needle electrodes. The experiment was designed (1) to correlate the amount of needle surface exposed to muscle tissue with the amount of voltage recorded and (2) to compare surface electrode recordings with needle electrode recordings made with the tip, 1 mm., 2 mm., and 3 mm. of the recording end exposed to muscle. The recordings were made from the left biceps muscle of seven subjects. The active electrodes (one surface and four needle electrodes with varying tip exposures) were placed in a circular pattern approximately ¾ inch from a centrally located surface reference electrode. The needle electrodes were inserted to an equal depth, but with different recording exposures, into the body of the muscle. The recording instrument was a six-channel polygraph* with integrators. Each subject contracted the biceps muscle five times by three different movements. Thus fifteen recordings were analyzed for each electrode and a total of seventy-five for each subject. Results indicated that (1) the surface electrode recorded the least voltage, (2) the needle electrode with 1 mm. of the tip exposed recorded the most voltage, (3) the surface electrode and the needle electrode with the tip exposed produced comparable voltages, and (4) similar electrodes recorded differently, depending on their position in or on the surface of the muscle.

76. Gingiva of Cattle.—*Iraida Piñeiro García, University of Puerto Rico, School of Dentistry.* A study of the gingiva of cattle was made from 525 color transparencies taken of the anterior teeth and surrounding structures of living animals. These revealed complete absence of stippling in the attached gingiva and of pigmentation in the free gingiva. Pigmentation in other parts of the gingiva varied from pink to purplish black to complete black. Histologic study was made of sections obtained from 26 animals 2 months to 11 years of age. The anterior portion of each mandible was divided at the mid-line; mesiodistal and labiolingual sections were obtained and stained with hematoxylin and eosin, Mallory's aniline blue, Gomori's silver stain, and Resorcein–Van Gieson stain. These showed parakeratotic basophilic stratified squamous epithelium more keratinized than the sole of the human foot; a granulated, nucleated stratum lucidum, one or two layers thick; distinct tonofibrils and intracellular granules in the prickle-cell layer; a large number of melanoblasts and cells with clear areas in their nuclei (giving them a ring-shaped appearance) in the basal layer; elastic fibers among the connective tissues in the lamina propria; epithelial attachment joined to the cementum because the crown, like the root, is covered by cementum; and gingival recession, associated with migration of the epithelial attachment toward the apex, progressing with age and unaccompanied by inflammation.

77. Localized Shwartzman Phenomenon in Buccal Pouch of Hamsters.—*Marvin M. Rosenberg, Department of Periodontology, Graduate School of Medicine, University of Pennsylvania, Philadelphia.* The study performed was concerned with determining whether the oral mucosa of the buccal pouch of hamsters was amenable to the localized Shwartzman phenomenon, which has previously been described only on cutaneous tissues. The lesion was produced by sensitizing a site on the mucosa of the buccal pouch with a submucosal injection of endotoxin isolated from *E. coli*, followed 14 hours later with an intraperitoneal eliciting injection of the same endotoxin. Within hours following the second injection, petechiae developed at the prepared mucosal site, which coalesced, producing a hemorrhagic lesion varying in size from 8 to 15 cm. in diameter. After 62 hours, necrosis was clinically evident, and the hemorrhagic necrotic lesion persisted for 1 week, at which time healing was evident. By 2 weeks the lesion was, for the most part, covered with epithelium. Healing continued up to the fourth week, at which time the mucosa appeared normal. Histologically, the response was primarily an inflammatory one found within the muscle layer subjacent to the mucosa and spread in a linear pattern. Necrosis of the underlying structures resulted

* Grass Model 5P3.

in abscess formation and ulceration, which subsequently organized and healed without scar formation.

78. Effectiveness of Single SnF_2 Topical Technic.—*Victor H. Mercer, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* Previous studies have shown that a single application of an 8 per cent solution of SnF_2 is a more effective anticariogenic technic than a series of four applications of NaF. However, in such studies no non-treated control group was used. It was considered important, then, to know the actual effectiveness of this technic when compared to a group receiving a single application of distilled water or to a group receiving a single application of NaF. Such a study was conducted in Pendleton, Indiana, where 1,000 children, aged six to seventeen years, were divided into four groups. Group I received a single application of distilled water; Group II a single application of a 2 per cent aqueous solution of NaF; Group III a single application of an aqueous 8 per cent solution of SnF_2; and Group IV two applications of an 8 per cent solution of SnF_2 1 week apart. All the applications were given by using the whole-mouth technic and by the clinical procedure previously described in detail. No radiographs were used, and all the treatments were conducted by means of portable equipment. The caries increments after 1 year for the four groups were as follows: for new DMF teeth, 0.81, 0.88, 0.40, and 0.35; for new DMF surfaces, 1.73, 1.85, 0.88, and 0.86.

79. Effect of NaF and CaF_2 on Salivary Glands in Mice.—*R. L. Capelle, R. C. Foster, Jr., and W. L. Griffin, Howard University, Washington, D.C.* Male and female Swiss mice in groups of five for each sex received NaF and CaF_2 in quantities of 10, 15, and 20 ppm for periods of 40 days via their drinking water. Control animals receiving tap and distilled water made up additional groups. Animals were weighed before, during, and at the termination of these experiments. Amounts of fluids consumed were measured daily. Upon sacrifice, salivary glands were removed at once and formalin-fixed. Sections were cut at 7μ and stained with hemotoxylin and eosin. For this report, only changes occurring at 10 ppm were considered. The most striking histologic changes as compared with the tap-water controls were as follows: (1) In animals receiving distilled water, narrower ducts, wider septa, fainter cell outlines, smaller and darker nuclei, and engorged vessels were observed. (2) In the group receiving NaF, acini had separated more, the septa were wider, and the ducts were not always patent. Some proliferation of ductal epithelium, fewer cells in acinar groups, more frequent cytoplasmic droplets and vacuoles, smaller nuclei, engorged vessels with breaks in the endothelium, and extravasation of red blood cells were also observed. (3) In the group receiving CaF_2, the acini showed large separations, the septa were very wide, and numerous ducts which were not always patent, with some proliferation of ductal epithelium, were observed. Frequent cell damage with numerous cytoplasmic droplets and vacuoles, smaller and darker nuclei, and vessels enlarged and engorged with some extravasation of red blood cells were also observed.

80. The Oral Retention of Various Foods.—*Donald G. Moon, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* The oral retention of different food substances was studied in order to determine the amount of variation in retention of the various foods in different individuals. The amount of carbohydrate in each test food was determined, and the amount of food remaining in the oral cavity was estimated by measuring the amount of carbohydrate in the oral washings following consumption of the test food through use of the anthrone method utilizing the Morris modified, Dreywood's anthrone reagent. Each sample of test food was analyzed, in order to compensate for possible variation in carbohydrate content from different samples. The foods tested were graham crackers, soda crackers, white bread, various brands of peanut butter, and bread with various peanut butters. Results indicated a variation in retention of the same test food by the same individual of approximately 5 per cent but a variation in the retention from individual to individual of the same

test food by approximately 12 per cent. The results obtained by these studies are in agreement with those previously reported by Bibby. However, the amount of variation in retention of a food sample in one individual and the variation in the retention of a food in different individuals was not so great as previously reported. This may be explained partly by standardization of the procedures used in this experiment and also by impressing upon the subjects the importance of following a regimented procedure in the collection of the retained food particles.

81. Casein-degrading Ability in Hamster, Rat, and Mouse Salivary Glands. —*John M. Shackleford, University of Alabama Medical Center, Birmingham.* Previous investigators have demonstrated the presence of proteolytic enzymes in the submaxillary glands of rat and mouse. The present study was undertaken in order to compare the activity of this enzyme in hamster salivary glands—a third rodent—with that of the rat and mouse. The parotid glands of hamster, rat, and mouse were included in this investigation because previous data of this nature had omitted the parotid portion of the salivary complex. Finally, an experiment to determine the effect of pilocarpine on tissue levels of this enzyme in hamster salivary glands was performed. Enzyme determinations on the supernatant of gland homogenates were made of each of the salivary glands under the same conditions of pH (9.5), temperature (53° C.), and substrate concentration (1 per cent casein). Proteolytic activity of the submaxillary glands was highest in the mouse, of intermediate value in the rat, and lowest in the hamster. In the case of the parotid gland activities, however, quite a different pattern was observed. Here the hamster gland exhibited the highest activity, the rat again had an intermediate value, and the mouse parotid showed little or no activity. Acute pilocarpine stimulation in hamsters produced a marked elevation in submaxillary gland proteolytic activity but had little or no effect on the parotid gland.

82. Studies concerning the Effects of Lysine Deficiency in the Rat.— *David K. Hennon, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* Previous workers have shown that when rats receive a diet deficient in lysine, there is a significant increase in dental caries. When a test meal of lysine was administered to humans, an increased production of gastric secretions occurred, notably

TABLE 1

EXPERIMENT I

Group	Animals per Group at End of Study	Mean Caries Score	Mean Initial Weight (Gm.)	Mean Final Weight (Gm.)	Per Cent Mortality
Control	20	9.0	55	256	0
Lysine-deficient	19	14.3	54	76	5
Lysine	15	9.2	56	152	25
Lysine+HCl	5	7.2	59	178	75
HCl	11	9.6	46	67	45

of pepsin and hydrochloric acid. It was considered important to test these latter observations by administering known amounts of sodium hydroxide or hydrochloric acid to rats, and study the effect of such a procedure on dental caries and skeletal dynamics. Two experiments were performed. The first tested the effect of hydrochloric acid (Series I) and in the second the effects of hydrochloric acid and sodium hydroxide (Series II) were compared. Series I is described in Table 1, and Series II in Table 2. The control animals received a stock corn diet, but all other groups received a lysine-deficient diet by substituting thoroughly browned, powdered whole milk for untreated powdered whole milk in the control diet. As the lysine supplement, 75 mg/ml of an aqueous

solution of L-lysine monohydrochloride was used; 1.0 ml of 0.05 N HCl was used as the HCl supplement; and 1.0 ml of 0.1 N NaOH was used for the sodium hydroxide supplement. The effect on weight gain and dental caries is shown in Tables 1 and 2.

TABLE 2

EXPERIMENT II

Group	Animals per Group at End of Study	Mean Caries Score	Mean Initial Weight (Gm.)	Mean Final Weight (Gm.)	Per Cent Mortality
Control	21	5.9	74	255	18
Lysine+HCl	23	8.4	74	90	23
HCl	14	10.0	74	60	53
Lysine+NaOH	14	9.2	74	90	53
NaOH	14	9.2	74	70	53

83. BIOGENESIS OF LIPIDS IN THE GINGIVA.—*Burt D. Stern and Jose Rabinowitz, Department of Periodontology, Graduate School of Medicine, University of Pennsylvania, and Veterans Administration Hospital, Philadelphia.* This project attempted to determine the rate of lipid biosynthesis in normal and in inflamed gingivae, as well as in the gingivae of subjects receiving therapy for non-dental pathology and which might be expected to induce change in metabolism. Uniform volumes of gingivae were incubated with 1 mg. each of TPP, DPN, AMP, and streptomycin and 0.5 mg. radioactive sodium acetate in phosphate buffer, pH 7.2, containing small amounts of magnesium chloride and nicotinamide for 2 hours under oxygen tension with constant mechanical shaking. Following incubation, an ether extraction was performed. Distilled water and unlabeled cholesterol, palmitic acid, and oleic acid carriers were added to the buffer phase. Following extraction, the ether was taken to dryness. Glacial acetic acid was added, and the lipids were desiccated under vacuum to wash out bound acetate substrate. The lipid residue was dissolved in absolute ethyl alcohol. Cholesterol was precipitated as the digitonide. Following centrifugation, the mother liquor was decanted. Digitonide was washed successively in absolute ethyl alcohol, acetone, and anhydrous ether, dissolved in methyl alcohol, transferred to a weighed aluminum planchet, taken to dryness, and counted. The fatty acid solution was taken to dryness. The residue was dissolved in chloroform, washed three times with distilled water, transferred to a weighed aluminum planchet pretreated with a detergent, taken to dryness, and counted. With appropriate calculations, results were obtained in counts per minute per milligram carbon yield. Blanks, with non-viable gingiva incubated, were carried out periodically to check and maintain technic standards of purity. Utilizing this technic *de novo*, biosynthetic cholesterol, palmitic acid, and oleic acid were obtained labeled with C-14 tag. This indicated that biosynthetically the removed gingiva was enzymatically intact for these synthetic processes. Statistical evaluation will be possible when a large enough number of samples has been assayed.

84. TRANSPLANTATION OF WALKER 256 CARCINOSARCOMA CELLS IN PULP AND PERIODONTIUM. *Donald E. Ore, University of Illinois College of Dentistry, Chicago.* The potential of the dental pulp as a site of primary and secondary tumor growth was studied. The investigation was divided into two phases: in phase 1 a suspension of virulent W256 cells was placed in coronal pulp exposures in the maxillary first molar of young rats and sealed with zinc oxide and eugenol; in phase 2 a suspension of virulent W256 cells was transplanted into the periodontal membrane. Controls of phase 1 consisted of dead W256 cells introduced into the pulp and sealed with zinc oxide and eugenol, and pulp exposures sealed with zinc oxide and eugenol. Histologic

examination at 2, 4, 8, 12, 14, and 21 days postoperatively indicated that in phase 1 no tumor proliferation as such occurred in the pulp, but by 12 days the pulp had become completely obliterated with osteoid material. Phase 1 controls showed osteoid material confined to the coronal portion of the pulp, with degeneration of the remainder of the pulp. Phase 2 showed marked tumor proliferation in the periodontal membrane and invasion of the pulp through the apical foramina by 21 days. No osteoid production was noted in this group. The results of this investigation indicated that a primary tumor "take" did not occur in the pulp, and that there was marked osteoid reaction when W256 cells were transplanted into the pulp. Whether this was a result of operative trauma, the presence of W256 cells, or both was not determined. Phase 2 indicated that the pulp will support the growth of tumor cells when they enter the apical foramina.

VIII. MICROBIOLOGY II. MARCH 18, AFTERNOON, SECTION A.

85. THE EFFECT OF FRACTION II OF HUMAN GLOBULIN ON LACTOBACILLUS.—*Thomas L. Grisamore and Patrick D. Toto, Loyola University School of Dentistry, Chicago.* It has been found that fraction II of human globulin in dilution of 0.33 gm. per cent, equivalent to normal blood concentration, and 0.165 gm. per cent completely inhibit the growth of a strain of *Lactobacillus* from a clinically positive case of dental caries and that further dilutions to 0.0165 gm. per cent produce partial inhibition of growth. What mechanism may be responsible for this activity? Microscopic agglutination tests were done, using hanging-drop preparations with two loopsful of saline suspension of *Lactobacillus* to which was added two loopsful of various dilutions of fraction II human globulin in saline solution. These were examined after 30 minutes at 37° C. A macroscopic agglutination test was done with 1 cc. of saline suspension of *Lactobacillus* to which was added 1 cc. of 10 mg. per cent fraction II human globulin saline solution. This was allowed to stand overnight at 37° C. The mixture was centrifuged, the supernatant discarded, and the centrifugate washed twice with saline solution. Some of the final centrifugate was fixed on a microscopic slide, covered with two drops of the fluorescein conjugate of antihuman globulin rabbit serum, and allowed to stand 15 minutes in a moist chamber. The slide was washed in saline solution, dried, and examined under ultraviolet microscopy. In the microscopic agglutination tests, agglutination occurred at final globulin dilutions of 10.5, 2.5, and 1.25 mg. per cent. The greatest dilution was equivalent to serum dilution of approximately 1/250. The agglutinated bacteria treated with the fluorescein conjugate of antihuman globulin rabbit serum showed fluorescence under ultraviolet microscopy.

86. A STUDY OF THE HEXOSAMINE METABOLISM OF *Lactobacillus casei*.—*H. Blumenthal, T. Shiota, M. P. McCann, and M. Disraely, National Institute of Dental Research, National Institutes of Health, Bethesda.* Hexosamines are known to be constituents of salivary mucoproteins and mucopolysaccharides. What role, if any, the oral flora may play in their metabolic breakdown is therefore important. With this in mind, a number of bacterial species was screened for growth in a medium in which the sole source of carbohydrate was either glucosamine or galactosamine. Of these, *Lactobacillus casei*, ATCC 7469, was chosen for further study. *Lactobacillus casei* which had been grown on galactosamine was transferred into media containing glucose, glucosamine, or galactosamine as the sole carbohydrate, and their growth rates were studied. As controls, cells grown in the presence of glucose rather than galactosamine were used. In the case of the galactosamine-grown cells, the organisms grew with equal rate on each of the three substrates. However, growth on galactosamine was approximately 50 per cent of that for the other substrates on a molar basis. For the glucose-grown cells, growth on glucose and that on glucosamine were almost identical except for a slight lag phase in the case of glucosamine. Galactosamine, however, was a very poor substrate under these conditions. Experiments with resting cells were then carried out to measure ammonia liberation and lactic acid formation from the hexosamines, and the following values were obtained. For cells grown on glucose, glucosamine, or

galactosamine with 5 micromoles/ml of glucosamine as substrate, ammonia production was 4.62, 5.08, and 2.28 micromoles/ml, respectively. When galactosamine was the substrate, the ammonia values were 0.88, 3.08, and 3.8 micromoles/ml. Corresponding lactic acid production was 4.3, 4.74, and 2.2 micromoles/ml with glucosamine as substrate, and 0.21, 2.14, and 2.47 micromoles/ml with galactosamine. The foregoing data suggest that *L. casei* can utilize the hexosamines as a source of both carbon and energy.

87. Studies on the Kinetics of a Salivary *Lactobacillus bactericidin.*—*Bernard J. Zeldow, University of Washington School of Dentistry, Seattle.* A *Lactobacillus bactericidin* found in both human parotid and submaxillary saliva has been described (*J. D. Res.*, **38**:798, 1959). Continuing studies on the kinetics of this antibacterial system, using the assay organism *Lactobacillus acidophilus*, ATCC 4357, are presented. To determine the effect of the saliva on the organism, viable counts were made by plating suitable dilutions at various intervals of time ranging from 0 to 24 hours. The growth of the assay organism in the control culture increased logarithmically. In contrast to this, the organism when grown in the presence of saliva containing 1–16 bactericidin units per milliliter rapidly lost its viability. This occurred after an initial period of multiplication, amounting to 1 or 2 generations. Such a trend is highly suggestive of "unbalanced growth." Moreover, it was found that, whereas half a bactericidin unit per milliliter halted multiplication temporarily, a quarter of a bactericidin unit per milliliter demonstrated no deleterious effect upon the organism. Saliva when dialyzed also lost its ability to inhibit the organism. By utilizing Warburg's indirect method for measuring acid formation, it was determined, furthermore, that the lethal action of saliva on this *Lactobacillus* was not due to interference with the glycolytic process.

88. Some Effects of Fluoride on the Metabolism of Salivary Bacteria.—*G. N. Jenkins, Department of Physiology, Medical School, King's College, Newcastle upon Tyne, England.* When saliva, at an initial pH of about 5.0, is incubated for 4 hours with sugar and more than 6 ppm of fluoride, the pH *rises*, whereas in the fluoride-free control the pH falls rapidly. This result establishes that salivary acid production is more sensitive to fluoride at pH 5 than at neutrality. The nature of this rise in pH has been investigated. The main factor has been found to be the inhibition of lactic acid production, leaving unopposed the alkali-producing mechanisms. The removal of lactic acid, presumably by oxidation, also occurs, and this reaction is accelerated by fluoride at pH 5 but not at 7. To determine whether fluoride has any direct effect on the alkali-producing systems, saliva was adjusted to pH with HCl and incubated with and without fluoride in the absence of added carbohydrate (i.e., no lactic acid was added, and little or none could be formed). The results showed a slight but insignificant tendency for fluoride to accelerate the rise of pH which occurs under these conditions. The effect on alkali production seems, therefore, unimportant compared with the other actions mentioned. An acceleration of lactic acid removal at pH 5.0 might be of more significance in caries than an inhibition of acid production. Once the critical pH has been reached, inhibition of further production of acid may have little influence on decalcification, but its removal might shorten the duration of decalcification.

89. Microorganisms of Deep Dentinal Caries.—*Clarence P. Canby and George W. Burnett, Walter Reed Army Institute of Research, Washington, D.C.* An investigation was made of the microorganisms of deep dentinal caries. To minimize extraneous contamination from the oral cavity, samples were obtained only from carious lesions of extracted teeth which contained a reasonable bulk of decalcified but leathery dentin. Exacting procedures were then applied to obtain a culture sample which was not contaminated by the diverse flora of the superficial portions of the lesion. Samples were initially cultured anaerobically in chopped-meat and thioglycolate broths, followed by anaerobic isolation of the strains on thioglycolate and tomato-juice agars. Immediately upon anaerobic isolation, the strains were dried from the frozen state, to preserve them

for subsequent biochemical and immunologic studies. Occasional attempts at initial aerobic culture of such samples on tomato juice, thioglycolate, and SL agars and glucose and tomato-juice broths were often negative. By the anaerobic technic, the isolation rate was uniformly positive except in those instances when the sample was removed too far ahead of the advancing carious front. The organisms isolated were uniformly Gram-positive, non-motile, non-sporulating, aciduric, and acidogenic bacilli. The colonial and microscopic morphology and cultural characteristics of twenty-one isolates were then determined when they were cultured anaerobically and aerobically on tomato juice, thioglycolate, and blood agars, and in chopped meat and thioglycolate broths for comparison with 14 strains of seven long-established representative *Lactobacillus* species. Many of the newly isolated strains would not grow aerobically or grew poorly. The colonial stability of the anaerobic isolates was best maintained by anaerobic cultivation.

90. STUDIES ON ORAL VEILLONELLAE.—*M. A. Mazzarella and I. L. Shklair, Dental Research Facility, Great Lakes, Illinois.* Dental interest in the *Veillonella* group developed with the finding of their biochemical activity on lactate, pyruvate, oxaloacetate, malate, fumarate, succinate, tartarate, proprionate, and acetate substrates to produce carbon dioxide and hydrogen with a resultant rise in pH. This suggested that *Veillonella* could metabolize lactic acid in the mouth to constitute a biological defense to the progress of dental caries. To test this hypothesis, the prevalence of these organisms was studied in the saliva and dental plaque substance of extreme caries-rampant (DMF over 15) and caries-immune (DMF = 0) groups with an average age of eighteen years. The group mean values are given in the table. The caries-rampant groups showed

POPULATION OF VEILLONELLAE

	Caries-immune	No.	Caries-rampant	No.
Per ml. whole saliva	6.0×10^6	35	1.4×10^7	33
Per μg. dry plaque	1,658	10	47,665	17

significantly higher populations of *Veillonella* in both saliva ($P = 0.005$) and plaque substance ($P = 0.006$) when compared to the caries-immune groups. Possibly *Veillonella* may function biologically to increase the buffering capacity of dental plaque. However, the larger population found in the caries-rampant group can probably be considered a direct result of the greater amount of lactate formed in these plaques, which can then serve as an essential substrate for the *Veillonella* group.

91. BIOCHEMICAL STUDIES OF ORAL FUSOBACTERIA.—*Robert R. Omata and Robert C. Braunberg, National Institute of Dental Research, National Institutes of Health, Bethesda.* In a study of the taxonomy and the biochemistry of the oral fusobacteria, over twenty-five strains were isolated from normal human subjects and several patients with Vincent's infection. Biochemical reactions were correlated with morphologic and colonial characteristics to establish specie differentiaton. The end-products of glucose fermentaton were separated and identified by paper-partition chromatography. Three main species or types were identified. *Fusobacterium nucleatum* and *F. polymorphum* were very similar in their biochemical reactions, both types producing indole and hydrogen sulfide, while acetic and butyric acids were the main products of glucose fermentation with terminal pH of 5.0–6.0. The morphology of these two types was dissimilar, while the colonial characteristics were quite similar. The third species, *F. fusiforme* (*F. plauti-vincenti*) did not produce any hydrogen sulfide or indole and fermented glucose to lactic acid with a terminal pH of 4.8–5.2. The colonial characteristics and cell morphology appeared to be different from the other species.

92. Experimental Intracutaneous Fusospirochetal Infections.—*Edward G. Hampp, Stephan E. Mergenhagen, and Robert R. Omata, National Institute of Dental Research, National Institutes of Health, Bethesda.* The oral fusobacteria and spirochetes have for many years been considered of etiologic significance in ulcerative gingivo-stomatitis. The experimental elicitation of infections in animals with pure cultures of these micro-organisms, either separately or in combination, has been lacking until recently when spirochetal abscesses were successfully produced in rabbits (Hampp and Mergenhagen, *Bacteriol. Proc.*, **1959**: 101, 1959). These studies were extended to include *Fusobacterium nucleatum* and *F. polymorphum*, both separately and in combination with the small oral treponeme and *Borrelia vincentii*. Intracutaneous lesions were routinely produced in rabbits with the fusobacteria which terminated in abscess formation in 24–48 hours and have existed up to 1 month. Similar lesions were elicited with heat-killed cells. Exotoxic activity was not demonstrated in the supernatant culture fluids of these micro-organisms. When a given strain of fusobacteria and spirochetes were cultured together or grown separately and recombined, the lesions subsequently produced by these synergistic combinations were more fulminating and grossly larger than when either organism was used alone. This was particularly evident in the former instance. Microscopically and culturally, it was found that the fusobacteria invaded from their individual lesions to spirochete lesions in their proximity on the same side of the animal. Further, an anachoretic effect was elicited when fusobacteria were introduced intravenously 24–48 hours after the initiation of intradermal spirochete abscesses, and the localization of the fusobacteria in these lesions produced a synergistic effect. However, culturing intravenously injected spirochetes from established fusobacterial lesions has not been successful up to this time. Histopathologic and cultural studies were conducted on all lesions throughout the various phases of these experiments.

93. Endotoxins from Oral Bacteria.—*Stephan E. Mergenhagen, Edward G. Hampp, and Henry W. Scherp, National Institute of Dental Research, National Institutes of Health, Bethesda.* Tryptic digestion of acetone-dried, Gram-negative oral bacteria (*Veillonella, Fusobacterium, Selenomonas*) released soluble endotoxins which elicited the dermal Shwartzman reaction. Oral diphtheroid bacilli and anaerobic streptococci did not yield Shwartzman-reactive endotoxins after similar treatment. In addition, acetone-dried veillonellae were fractionated by the two-phase phenol-water procedure of Westphal as applied by Tauber (*J. Biol. Chem.*, **234**:1391, 1959). Acetone and sodium chloride precipitated from the aqueous phase a highly toxic fraction (LP) containing lipopolysaccharide-like substances and nucleic acid components amounting to 3.5 per cent of the dry weight of cells; ethanol precipitated from the phenolic phase denatured proteins (PR) amounting to 11.3 per cent. Both fractions were soluble on treatment with 0.05 N sodium hydroxide and subsequent neutralization. LP contained 10 per cent Kjeldahl nitrogen, 4 per cent protein (Folin-Ciocalteu method with bovine albumin as standard), and 4.2 per cent phosphorus; absorbed strongly at 260 mμ, indicating nucleic acid components; gave a strong Molisch test for carbohydrate; and yielded 5.6 per cent of chloroform-soluble material after acid hydrolysis. PR contained at least 90 per cent protein and reacted negatively to the Molisch test. The LD_{50} of LP injected intraperitoneally into Swiss Webster mice was 1.0 mg.; as little as 30 μg produced inflammatory necrotic lesions in the skin of New Zealand albino rabbits and as little as 4 μg in prepared sites in rabbit skin for the local Shwartzman reaction. Comparable ranges of doses of PR were not toxic.

94. Incidence of Oral Group D Streptococci.—*A. N. Bahn, I. L. Shklair, M. Mazzarella, and J. C. Calandra, Northwestern University Dental School, Chicago, and Dental Research Facility, Great Lakes Naval Training Center, Great Lakes, Illinois.* Saliva samples from dental clinic patients and naval recruits were examined for Lancefield group D streptococci. One milliliter of each saliva sample was placed in a phenol-red sodium azide enrichment broth containing 1 per cent dextrose. From the

positive samples, one loopful was inoculated into ethyl violet azide broth. The positive samples from this screening broth were streaked on mitis-salivarius tellurite agar and an ethyl violet azide medium containing agar and 2,3,5-triphenyl tetrazolium chloride. Those isolates resembling enterococci on these selective media were inoculated into pH 9.6 broth, 6.5 per cent NaCl, 0.1 per cent methylene blue milk broths, and a 40 per cent bile medium. Those growing in these isolation media were then grown in tryptose phosphate or Todd-Hewitt broths. The bacteria were harvested, and antigens were prepared by the Lancefield or Rantz-Randall method from these positive isolates. Those demonstrating a positive precipitin reaction with group D antiserum and showing growth at 45° C. were speciated by biochemical tests. Of 303 salivas of dental clinic patients analyzed to date, 70, or 23.1 per cent, showed some group D streptococci. In contrast, of 443 naval recruits tested, only 3.6 per cent showed enterococci. However, 64 naval recruits were tested during an epidemic of an infection with an upper respiratory virus, and 34.1 per cent had group D streptococci. No correlation can yet be made between the presence of the group D streptococci and oral disease.

95. Serological Characterization of *Streptococcus mitis*.—*M. Strauss, J. E. Nygard, A. N. Bahn, and J. C. Calandra, Northwestern University Dental School, Chicago.* Antigenic analysis of *Streptococcus mitis*, the alpha hemolytic streptococcus frequently isolated from the oral cavity, was made to ascertain the nature of the typing antigens and to determine the Lancefield-grouping antigen. Whole-cell and cell lysate–immunizing antigens of American Type Culture Collection strains 9811, 6249, and 903 of *S. mitis* were injected intravenously and with Freund's adjuvant subcutaneously into rabbits. The antisera were collected and tested with antigens prepared by the Lancefield and Rantz-Randall methods. Antigens of the above strains and commercially prepared antigens of Lancefield groups D,G,K,L, and M were used to test the antisera by the capillary-tube precipitin test. The antisera prepared from the lysed cells and injected subcutaneously gave the highest precipitin titers. All strains showed considerable cross-reaction. Recent reports of alpha hemolytic streptococci reacting with Lancefield group M were not confirmed. None of the antisera reacted with antigens from Lancefield groups D,G,K,L, and M. Studies are now in progress to test oral isolates of *S. mitis* with these Lancefield groups. A serological characterization of this important oral streptococcus will allow a better means of studying this inadequately classified micro-organism.

96. The in Vitro Inhibition of Beta Streptococci by an Oral Streptococcus.—*Henry A. Bartels, Harry Blechman, and Diana Lorieo, Murry and Leonie Guggenheim Foundation, Institute for Dental Research, College of Dentistry, New York University.* An anhemolytic streptococcus, closely resembling *Streptococcus salivarius* biologically, was isolated from a sample of whole stimulated saliva. The isolate exhibited anti-beta streptococcal activity on both pour and streak plates. Zones of inhibition equivalent to 4–6 mm. were produced around single colonies of the anhemolytic streptococcus. Surface growth of beta streptococci was completely inhibited when the anhemolytic streptococcal population of a pour plate approximated 8,000 colonies. Sterile filtrates showed variable activity, depending on the type of filter used and the nature of the test medium. The anti-beta streptococcus activity was apparently not associated with biogenic hydrogen peroxide or acid production by the isolated streptococcus strain. The substance responsible for the antibacterial activity described was found to diffuse through a cellophane dialyzing membrane and retained its activity in a solid-phase medium for at least 7 days.

IX. NUTRITION AND METABOLISM. MARCH 18, AFTERNOON, SECTION B

97. Food Requirements in the Antarctic.—*Robert J. Adams and William R. Stanmeyer, U.S. Naval Medical Research Laboratory, New London, Connecticut.* The effect of the stresses of the Antarctic environment upon personnel in terms of food requirements was measured monthly for 10 consecutive months in 109 men stationed

at Little America V. The mean age of these men was 28.3 years, and the daily work required of them ranged from the sedentary occupation of a radio operator to the vigorous efforts required in shoveling snow into snow melters. It was felt that the intense cold, prolonged periods of daylight and darkness, food palatability after prolonged storage, in addition to the psychosomatic factors of the isolation of Antarctic life, might affect not only caloric intake but food preferences as well. Monthly observations on food consumption expressed as (*a*) calories, (*b*) protein, (*c*) fat, (*d*) carbohydrate, (*e*) water, and (*f*) inorganic ions of calcium, phosphorus, and iron were recorded. Working outdoors in the bitter climate during the daylight months resulted in loss of body heat through breathing and skin surface radiation, elevating the daily caloric intake to a mean of 4,842 per day per man. The daily carbohydrate intake was increased from a mean of 379 gm. per day per man to a mean of 588 gm. per day, a total increase of 55 per cent. In like manner, protein consumption increased 100 per cent and consumption of foods containing phosphorus and iron increased with similar proportions. Input of fats and calcium-containing foods showed little change. During the winter dark months, when the camp activity centered indoors, the daily caloric intake fell to a mean of 4,068 per day per man, while carbohydrate consumption decreased to a mean of 539 gm. per day per man. Even this represented an increase of 43 per cent above intakes considered normal for this age and work group.

98. Ascorbic Acid Plasma Levels and Oral Health in Personnel Wintering-Over in Antarctica.—*Max J. Perlitsch, Arne G. Nielsen, and W. R. Stanmeyer, U.S. Naval Medical Research Laboratory, U.S. Naval Submarine Base, New London, Connecticut.* The ascorbic acid plasma levels (AAPL) of 26 men were determined monthly and correlated with their oral health as reflected by monthly PMA and Russell indexes. The subjects of the study were personnel, ranging in age from twenty-one to thirty-seven years, that wintered-over in Antarctica during the IGY. Of the 26 men studied, 13 were indoor workers and 13 were outdoor workers. The average AAPL for the year of the indoor group ranged from approximately 0.6 to 1.2 mg. per cent. This range falls within what is generally accepted as normal. However, the average AAPL for the outdoor group over the same period ranged from approximately 0.5 to 0.7 mg. per cent, which is generally accepted as low normal. Examination of the data on a month-to-month basis revealed that the average monthly AAPL of the indoor group remained relatively constant throughout the year. The average monthly AAPL of the outdoor group showed a progressive and statistically significant decline for the year from that of the indoor group. To correlate oral health with the AAPL, both groups were examined monthly by the same investigator to determine the PMA and Russell indexes. In the analysis of the PMA data, the intensity factor was initially disregarded, and only total number of areas of inflammation considered. Then the PMA data were reinterpreted in terms of intensity of inflammation alone. On the basis of these two approaches, correlation of the AAPL and PMA findings for both groups revealed no statistically significant relationship. The clinical data from the above study, which included the monthly charting of periodontal pocket depth and full-mouth roentgenographic surveys taken at 6-month intervals, are being examined to determine the relationship of the ascorbic acid plasma levels to periodontal findings.

99. DNA Metabolism of Basal-Cell Layer in Mouse.—*William A. Schoenheider, Jr., Loyola Dental School, Chicago.* The synthesis of DNA by epithelial cells is an index of the cell population turnover. We wished to study the DNA synthesis of the basal-cell layer of the oral mucosa of the mouse. Twenty 300-day-old mice developed by a cross of C57 and Black were injected with 50 μc of radioactive thymidine. They were sacrificed at intervals of 15, 30, 45, and 60 minutes and 2, 4, 12, 24, 44, 46, 48, and 50 hours. The soft-tissue specimens (tongue) were cut at 3μ and stained by the Feulgen method for DNA. The nuclei which synthesized the radioactive thymidine gave off β radiation. The mounted stained sections were used to expose small squares of stripping emulsion for 30 days. The slides were developed and covered using

Canada balsam. The number of labeled nuclei located in the basal layer was counted in percentage for each time interval. The labeled nuclei were first observed on the dorsum of the tongue at 15 minutes. The maximum percentage, 17.10, was observed at 12 hours. The count slowly decreased until, at 50 hours, only 0.15 per cent was found. The ventral surface of the tongue presented a similar type of curve, starting at 15 minutes and ending at 50 hours, the maximum percentage reached at 12 hours being 14.50.

100. Dietary Changes and Metabolic Adaptations.—*David Bixler, Grace Kilsheimer, J. Ashmore, and J. C. Muhler, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* During the past few years it has become evident that changes in the carbohydrate, protein, or fat composition of the diet will cause marked changes in certain biochemical parameters of hepatic metabolism of rats maintained for several days on a given diet. For example, it has been demonstrated by Friedland and Harper that replacement of the major carbohydrate content of stock diets with either protein or fat results in an increase in hepatic glucose-6-phosphatase activity. Such an effect has been considered a metabolic adaptation to an increased need for hepatic glucose production. In the present study the effect of diet on changes in hepatic metabolism was compared in chicks and rats. While high concentrations of fat (60 per cent) are required to produce increases in hepatic glucose-6-phosphatase activity in rats, a marked increase in the activity of this enzyme was found in chicks fed on a diet containing only 20 per cent fat. Although the increase in phosphatase activity in the rat is transitory and has been observed only during the first week on the diet, the effect in chicks persisted for as long as 16 days. The increased phosphatase activity observed in chicks fed on high-fat or high-protein diets was associated with an increase in adrenal weight. It is suggested that changes in hepatic glucose-6-phosphatase activity induced by dietary change may be mediated through changes in activity of the adrenal cortex.

101. Metabolism of Citric Acid by the Rabbit Mandible.—*R. Van Reen, Dental Division, Naval Medical Research Institute, National Naval Medical Center, Bethesda.* Citric acid occupies a central position in carbohydrate metabolism, and, through the aerobic degradation of this and subsequent compounds and through the electron transfer reactions, cells obtain the greater portion of the energy required for numerous endothermic reactions. Citric acid metabolism in calcified tissues is of particular interest, since these tissues accumulate citric acid. Our laboratory has recently indicated that this accumulation cannot be explained on the basis of previous suggestions that there is a lack of isocitric dehydrogenase in calcified tissues and thus a metabolic block at this point in the tricarboxylic acid cycle. High levels of isocitric acid dehydrogenase activity were found in the epiphysial, metaphysial, and marrow elements of the femur, and these levels exceeded the activities of aconitase. In the present work, these studies were extended to the mandible of the rabbit. The bone was divided into four sections consisting of the relatively thin central portion of the ramus, the peripheral thickened areas of the ramus, and the inferior and superior portions of the body of the mandible. The dentition was removed before assays were made. As in the case of the femur, it was found that the various sections of the mandible contained considerable isocitric dehydrogenase activity and that this activity exceeded the aconitase activity in all sections. It is of considerable interest that the greatest activity of aconitase and isocitric dehydrogenase was found in the peripheral areas of the ramus.

102. (Not presented.)

103. Nutritional Studies on Desalivated Rats.—*Edgar M. Wagner, David Bixler, Joseph C. Muhler, and William G. Shafer, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* Repeated studies in this laboratory have demonstrated that animals which have had their major salivary glands surgically

removed do not eat as much, or gain weight as rapidly, as do comparable unoperated animals. Shaw has demonstrated that force-feeding eliminates such weight-gain and food-consumption discrepancies. In this study the effect of force-feeding a special diet compounded in this laboratory to desalivated and control animals was studied. Weanling Wistar rats were divided into three groups: desalivated and force-fed; control and force-fed; and control fed ad libitum. The diet was composed in per cent of the following ingredients: casein, 20; *l*-cystine, 0.3; Dextrin, 66.7; corn oil, 8.0; salt mixture, 4.0; vitamin mixture, 1.0; vitamins A and D, 0.3 gm/kg diet; vitamin E, 50 mg/kg diet. One gram dry diet was diluted with 1.3 ml. water and injected through polyethylene tubing into the stomach twice each day. Body weights were recorded each week. Animals from each group were sacrificed after experimental periods of 0, 20, 40, and 60 days. Total body nitrogen analysis (micro-Kjeldahl) and fat analysis were performed on each animal. The data indicated that force-feeding with this diet produced good metabolic balance and growth in control animals and eliminated weight and nitrogen metabolism differences between control and desalivated rats.

104. Effect of Pantothenic Acid Deficiency on Peridental Collagen.—*Walter L. Gabler, Northwestern University Dental School, Chicago.* Because of its relationship to coenzyme A and glycine, it was thought that pantothenic acid deficiency might produce some effect on the collagen in the peridental structures and on the rate of wound healing. Twenty of a group of 40 male rats weighing 150 gm. at the start were placed on a diet deficient in pantothenic acid and were given a pantothenic acid inhibitor orally. The 20 others were fed a standard rat cube diet. When the deficiency state was evident, gingival tissues of rats in each group were traumatized with a sharp instrument, and, at the same time, an incision was made in the backs and closed with sutures. Animals were sacrificed at 3, 5, and 7 days thereafter. Tissues taken from the backs of the pantothenic acid–deficient animals were grossly thinner than those from the normals. Tearing tests of healing tissues were not different between groups until the fifth postoperative day. Then the pantothenic acid–deficient specimens tore with less weight applied. Histologic sections through the wound sites showed little difference between the two groups. Emulsified healed connective tissue and acrylic sections through healed incisions, studied by means of the electron microscope, showed no differences in collagen fibers. Radiographs of the jaws and teeth of both groups showed significant periodontal change in the traumatized regions, but the deficient and normal groups seemed almost alike in extent of damage.

105. Protein Deprivation in Rats.—*Henry M. Goldman, Boston University.* Experiments have been conducted concerning the maintenance of young rats on (1) lysine-free diets; (2) lysine-supplemented controls; (3) protein-free diets; (4) complete withdrawal of food; (5) highly restricted diets; and (6) mimosine, an analogue of dehydroxyphenylalamine. Reported herewith are the findings of the animals on protein-free diets. The physical progress of these animals was noted. The control animals sacrificed at the end of 4 weeks had approximately doubled their weight, whereas the experimental group had added 20 gm. on the average. By the time the 6-week group had been reached, all the experimental animals weighed less than they had at the time of the start of the experiment. The changes in the experimental rats were characterized by a loss of weight, scrawniness in appearance, and a marked weakness. The microscopic changes within the periodontium of the experimental animals were quite noticeable and became progressively more severe in later stages of the experiment. The alveolar bone evidenced osteoporotic changes, in some areas considerable osteoclastic activity and in other areas a rather significant absence of such activity. The latter was interpreted as a change produced by the failure of apposition of new bone following the resorption of the pre-existing bone as part of a normal activity. The cementum in some cases exhibited considerable resorption and in other cases lesser resorptive changes. The periodontal membrane exhibited degenerative changes, such as disorganization of the membrane, with some areas exhibiting a complete loss of

identity of the principal fibers. In some cases the fibers which did persist ran in a completely parallel direction to the root surfaces.

106. (Not presented.)

107. THE PATTERN OF EXCHANGE OF MAGNESIUM^{-28} BETWEEN PLASMA AND BONES IN THE ALBINO RAT.—*Parker E. Mahan, Terrance A. Rogers, and Leslie R. Burrows, Department of Dental Research, University of Rochester School of Medicine and Dentistry, Rochester, New York.* Twenty-two mature female rats were injected with magnesium^{-28} and were sacrificed at intervals up to 24 hours. The specific activities of magnesium in plasma and three different bones were determined. The equilibration of bone magnesium with that of plasma showed a rapid increase during the first 3 hours and a slower, exponential increase for the duration of the experiment. The turnover times of the slow, exponential increase in rib, calvaria, and femur were found to be 76.9, 73, and 153.8 hours, respectively. The percentages of bone magnesium equilibrating rapidly in rib, calvaria, and femur were 2.7, 6.5, and 5.7, respectively. At the end of 24 hours the magnesium of the rib, calvaria, and femur was 4.3, 11.9, and 8.2 per cent equilibrated with plasma magnesium, respectively. A comparison of the pattern of exchange of magnesium observed here with that seen in similar experiments on soft tissues was made. It was tentatively concluded that the early, rapid phase of equilibration represents mainly the exchange of magnesium in the cellular elements of the bones and that the slow phase represents mainly equilibration of the magnesium associated with the mineral component of the bones.

108. RADIOCALCIUM UPTAKE IN CALCIFIED TISSUES OF THE RHESUS MONKEY.—*Peter K-J. Yen and James H. Shaw, Harvard School of Dental Medicine, Boston.* To determine how much calcium within calcified and soft tissues was exchangeable at 5-, 27-, 51-, and 168-hour intervals, 3 male rhesus monkeys were injected intravenously with 8–12 mc. Ca^{45} as $CaCl_2$ in isotonic saline and sacrificed 168 hours later. Appropriate samples were collected at each interval. Ca^{45} radioactivity and total Ca^{40} in mEq per gram of tissue were determined for successive layers of calcified tissues, enamel, dentin, calvarium, and long bones; for soft tissues, parotid, submaxillary gland, kidney, liver, skin, and muscle; and for body fluids, blood, saliva, and urine. A ratio of 1:1 between the specific activity in the tissue and the specific activity in the blood serum indicated 100 per cent exchange, i.e., a complete equilibrium in soft tissues within 24 hours. After 168 hours, the ratios were as follows: endosteal layer of the calvarium suture, 1:15; calvarium, 1:25; enamel of unerupted teeth, 1:30; shaft of long bones and internal dentin of unerupted and erupted teeth, 1:50; dentin of erupted teeth, 1:500; and enamel of erupted teeth, 1:1,000. There was an exchange gradient in bones, the subperiosteal layer exchanging more rapidly than the inner layers in long bones, while the exchange was the reverse in the flat. First and second molars showed an increasing gradient from the internal toward the surface enamel and a marked decreasing gradient from internal dentin toward external dentin. There was also an increasing gradient in similar layers from the 5-hour samples toward the 168-hour samples.

X. CLINICAL STUDIES. MARCH 18, AFTERNOON, SECTION C

109. CLINICAL EVALUATION OF EFFICACY OF 2-CHLOROPROCAINE AND LIDOCAINE.—*A. L. Ingino and W. J. Carter, Children's Mercy Hospital and University of Kansas City School of Dentistry, Kansas City, Missouri.* This investigation compared the efficacy of 2-chloroprocaine with lidocaine. Patients were evaluated under clinical conditions by both objective and subjective methods. Procedures included the use of a standard electric pulp tester for determining the presence or absence of anesthesia, time of onset, and duration of anesthetic effect. Each patient was tested prior to anesthesia and graded at intervals of 15 seconds for onset and at 15-minute intervals for duration. Fifty-four patients were used in this study: 21 received lidocaine, and 33 re-

ceived 2-chloroprocaine. The results showed that the quality of nerve blocks produced by each agent was similar. The use of 2-chloroprocaine resulted in a shorter onset time and also a shorter duration. The average time of onset with 2-chloroprocaine was approximately 3 minutes, while that for lidocaine was 5 minutes. Duration for 2-chloroprocaine was approximately 1 hour, as compared to an excess of 2 hours for lidocaine.

110. An Objective Evaluation of a New Psychotropic Agent.—*N. B. Argerson, W. J. Carter, and B. K. Forscher, Children's Mercy Hospital and University of Kansas City School of Dentistry, Kansas City, Missouri.* This was a continuation study on the clinical evaluation of psychotropic drugs as premedication for apprehensive young dental patients. A double blind study was designed to test the efficacy of Kevadon(R) (N-phthalyl-glutamic acid imide) on 100 children ranging in age from three to fifteen years. Premedication was by oral route at approximately 6-hour intervals in the 24-hour period immediately preceding the dental appointment. Behavior and attitudes of the patients were observed by one of us (N. B. A.) before, during, and after each session of dental treatment. Subconscious apprehension in the patient was evaluated on the basis of changes in galvanic skin resistance measured in the palm of the hand. Each patient was studied once before premedication (drug or placebo) and once after premedication in the course of their routine visits to the clinic. The data indicate that the results obtained by use of this agent are similar to those previously reported obtained with psychotropic agents. It appeared that anxiety was affected differently from real and pronounced fear with this regimen of premedication. This is in keeping with other observations relative to the use of psychotropic agents.

111. Mast-Cell Density—Diphenylhydantoin Sodium Gingival Hyperplasia.—*C. O. Dummett and J. A. Ashhurst, Veterans Administration Hospital, Tuskegee, Alabama, and T. E. Bolden, Seton Hall University, Jersey City.* Gingival biopsies were taken from epileptic patients maintained on dilantin sodium therapy for periods varying from 3 to 11 years. One portion of the specimen was fixed in Helly's fluid and sectioned serially at 6 μ. Representative sections were stained with hematoxylin and eosin and with thionin. One portion was fixed according to the method of Smith and Atkinson (1956). Mast cells were counted in a given section of tissue stained either with thionin or according to the method of Smith and Atkinson. The surface area of the section was measured with a compensating polar planimeter at a magnification of 8 $\times$. The ratio of cells to surface area was then computed and recorded as cells per square millimeter of surface. Ten specimens were studied. Mast-cell density averaged 24.29. No apparent correlation existed between the amount of collagenous tissue, the degree of inflammatory infiltrate, and the mast-cell population. The period of administration of dilantin sodium therapy apparently did not influence the mast-cell population.

112. Cotton Fibrils in Dental Cysts.—*Herman Medak and J. P. Weinmann, University of Illinois College of Dentistry, Chicago.* The significance of hyaline bodies in dental cysts has puzzled oral pathologists since such bodies were first described by Rushton. They were found to occur in a low percentage of dental cysts submitted for diagnosis. We observed these bodies in eight cases of residual cysts and in one radicular and one follicular cyst. Common to our cases was a history of previous surgical intervention, dating about 1–15 years back. The bodies were found deposited in the lumen or in the lining epithelium. They resembled cotton fibrils seen in a surgical specimen containing parts of a sponge left behind after a mastectomy. Chemical tests for cellulose made on two specimens were positive. In one case the fibrils showed the typical birefringence of cotton fibers. In the remaining cases the fibrils showed swelling and calcification. However, in all cases, the appearance of a smaller or larger number of fibrils had remained unchanged, and the morphological resemblance to cotton fibers was unmistakable.

113. (Not presented.)

114. CONTROL OF POSTSURGICAL SEQUELAE WITH FIXED SURGICAL DRAIN AND/OR PHARMACEUTICAL AGENTS.—*Murray M. Hoffman, Chicago.* Preliminary double-blind clinical studies with adequate placebo controls, utilizing advocated standard oral dosages of (1) antihistaminics, (2) antispasmodics, (3) chlorpromazine (ataractic), (4) water-soluble citrus bioflavinoids, and (5) repository tubocurarine (intramuscularly), were checked against surgically fixed drain controls, in which pharmaceutical agents were or were not utilized, for pain, edema, trismus, ecchymosis, and hematoma. Over 600 office patients, aged fourteen to seventy-eight, who underwent complicated and non-complicated surgical removal of impacted mandibular third molars comprised this study. In 131 of these patients, polyvinyl drains were inserted through a prepared buccal sulcus fenestration and sutured *in situ* for 24–36 hours postoperatively. All patients either received 600,000 units of repository penicillin intramuscularly at time of surgery or were prescribed standard oral dosages of the broad-spectra antibiotics. Consequently, it was felt that infection as a contributory factor to inflammation was controlled. Oral temperature readings were taken daily for the first 5 days postoperatively. Control of the sequelae was optimum in those patients in whom the surgical fixed-drain procedure was combined with the pharmaceutical agents. Fixed-drain procedure alone was significantly more effective than the most effective pharmaceutical control. Of the latter, the order of effectiveness, when used alone, was (1) repository tubocurarine, (2) water-soluble citrus bioflavinoids, (3) chlorpromazine, (4) antihistaminics, and (5) anti-spasmodics. Three- to 5-day preoperative dosages followed by postoperative dosages were significantly more effective than a postoperative regimen alone. All test groups were significantly more effective than placebo controls.

115. EVALUATION OF PANORAMIC X-RAY PROCEDURES—*L. M. Kraske and M. A. Mazzarella, Dental Research Facility, Great Lakes, Illinois.* Modern roentgenographic procedures are slow because of the cumbersome number of small films handled during exposure and development. To accelerate X-ray procedures, there have been attempts to produce X-ray pictures of an entire dental arch on one single film and with one single exposure. Recently, members of the National Bureau of Standards and affiliated military service personnel developed an automatic extraoral apparatus which utilized principles of circular panoramic photography to produce an image of the entire oral region on a single film by means of a rotating cassette and X-ray head. For professional evaluation, an X-ray machine using these principles has been made available to the armed services. The "Panorex" has been found to produce, with ease and rapidity, a moderately acceptable, full-mouth X-ray picture. This resultant film showed grain and poor contrast, which caused difficulty in diagnosis of incipient interproximal caries and prevented outlining of lamina dura. However, the definition of gross lesions, restorations, and bone pathology was easily accomplished by the "Panorex" to demonstrate the general oral health of the individual. At this stage of the investigation (753 patients studied), it is felt that the "Panorex" film alone cannot replace the oral examination by a dental officer. However, it showed considerable merit for the supplementation of this examination, for positive identification in forensic procedures, and for permanently recording the general oral condition of the recruit on initial entry into military service for future Veterans Administration liability.

116. COMPARISONS OF THE CARIES EXPERIENCE BETWEEN IDENTICAL AND FRATERNAL TWINS AND UNRELATED CHILDREN.—*Robert C. Caldwell and Sidney B. Finn, University of Alabama School of Dentistry, Birmingham.* The use of matched twin pairs for testing caries-inhibiting agents was suggested because of their marked similarity in genetic and environmental factors. This study evaluated the dental caries experience of 35 pairs of identical twins, 31 pairs of fraternal twins, and 66 pairs of unrelated children matched only as to age, race, and sex but living in the same environment. The most critical comparison of caries experience between pairs is by a surface-to-surface and tooth-to-tooth evaluation rather than by absolute total differences. Identical twins had a mean DMF tooth difference of 4.54 ± 3.43 and DMF surface difference

of 12.25 ± 9.54. The fraternal twins had a mean DMF tooth difference of 5.87 ± 3.59 and DMF surface difference of 15.41 ± 9.55. The unrelated children had a mean DMF tooth difference of 6.45 ± 3.58 and DMF surface difference of 17.45 ± 7.57. In comparing the identical twins with the fraternal twins, there was no significant difference in either DMF teeth or DMF surfaces. In comparing identical twins with the unrelated children, both differences were significant ($0.01 < P < 0.005$). When comparing the fraternal twins with the unrelated children, the differences were not significant ($P < 0.1$). In comparing all twins with the unrelated group, the differences were significant ($0.05 < P < 0.01$). The coefficient of variation was highest for the identical twins (75.4 + 77.9) and lowest for the unrelated children (55.5 + 43.3). For fraternal twins it was 61.1 + 61.9.

117. Experimental Studies on the Mechanism of Mandibular Fractures.*—*Donald F. Huelke, University of Michigan Medical School, Ann Arbor.* Using a strain-sensitive lacquer,† the areas of high tensile strain in mandibles resulting from low-energy impacts to the mid-sagittal chin point were determined. The lacquer was sprayed on dry, degreased bones and allowed to dry for 15–20 hours. Upon impact, the lacquer cracked in response to the underlying tensile strain in the bone; the cracks first appeared in areas of highest tensile strain. Failure (fracture) occurred in these areas if sufficient load was applied. By analyzing the deformation patterns in the areas of high tensile strain, the role they played in the production of fractures was determined. Mandibles oriented so that the condyles were free to move under impacts behaved like pin-jointed arches. Impacts to the chin point—the center of the arch—flattened this area and deformed the rest of the arch by spreading the lateral alveolar walls, mental foramina, and subcondylar areas outward. If the ends of the arch were fixed (non-movable), the subcondylar area vibrated, bending both inward and outward, the other patterns were similar to those of pin-jointed arches. Various strain patterns and their correlation with clinical cases were presented and illustrated.

118. The Stability of Mandibular Rest Position.‡—*Judson C. Hickey, Benjamin H. Williams, and Julian B. Woelfel, College of Dentistry, Ohio State University, Columbus.* The stability of mandibular rest position was tested by two methods as measured on cephalometric roentenogram tracings. Two groups of dentulous subjects with random occlusions were selected. Group I was determined to be at rest position by electromyography alone, while Group II was determined by combined electromyographic and clinical means. The electrical activity of the temporal and digastric muscles was used to determine electromyographic rest position. This activity was measured simultaneously by (1) a pen-recording polygraph, (2) a cathode-ray oscillograph, and (3) a high-fidelity amplifier and speaker. Two roentgenograms were made during rest position for each subject on each of two sittings. The sittings were approximately 1 year apart. Thus a total of 4 roentgenograms of rest position was made for each subject and 44 roentgenograms for each group. Measurements between certain anatomic structures were used to determine the stability of the rest position (1) within the individual at the same sitting, (2) within the individual at the different sittings, and (3) between the two groups. The measurements were subjected to statistical analysis, which indicated (1) that the rest position was stable within the individual at the same sitting, (2) that the rest position was stable within the individual at different sittings approximately 1 year apart, and (3) that there was no significant difference between the methods tested. However, differences were of a magnitude to make the stability of rest position questionable for certain individuals. A better measurement

* This investigation was supported by Research Grant D-895 from the N.I.D.R., U.S. Public Health Service.

† Stresscoat, Magnaflux Corp., Chicago 31, Illinois.

‡ This investigation was supported by Research Grant D-548 from the N.I.D.R., U.S. Public Health Service.

system must be devised to indicate that small changes are solely a result of changes in mandibular rest position.

119. Statistical Variances in Electromyographic Analysis of Jaw Movements.*—*Julian B. Woelfel and Judson C. Hickey, College of Dentistry, Ohio State University, Columbus.* The objectives of this study were to determine by means of electromyography the similarities and differences occurring in a test group performing specific mandibular excursions and to provide data on the role played by the external pterygoid muscle in positioning the jaw. High-fidelity recordings were made on an eight-channel pen-writing electromyograph. All activity was referred to a multielectrode standard generalized reference. Recordings were made from ten dentulous subjects. Monopolar surface electrodes were secured over seven muscle areas. A monopolar needle electrode was inserted intraorally into the left external pterygoid muscle. After practice, the subjects performed each of 15 different learned jaw positions five times. Thus a total of 750 electromyograms was made, 75 from each subject, 50 for every jaw position. After careful study and measurement, these recordings from eight muscle areas were classified into twelve groups according to the magnitude and frequency of the pen lines. The tabulated data were subjected to statistical analysis for mean, standard deviation, and standard deviation of the measurement error. The results showed the range of variability of muscle response and indicated the fellowing: (1) the temporalis muscle was capable of both unilateral and fractional response; (2) the right and left digastric muscles did not function individually; (3) the masseter muscle showed very little activity during protrusions or lateral-directive jaw positions; and (4) the external pterygoid muscle was inactive for hinge or retruded openings of approximately 1 cm.

120. Threshold and Optimum Force Values for Maxillary Anterior Tooth Movement.—*Charles J. Burstone and Murray H. Groves, Jr., Indiana University School of Dentistry, Indianapolis.* A study was designed primarily to demonstrate the lowest possible force values that would retract anterior teeth by simple tipping if relatively continuous forces were applied. The relationship between force magnitude and rate of tooth movement was also investigated. Helical tension springs were employed to retract protrusive anterior teeth on children with a mean age of nine years. Twenty-two patients were included in the study. The initial force values from the tension spring ranged from 25 to 150 gm. per quadrant, with an average rate of 13 gm/mm. The degree of movement was determined from lateral head plates and specially designed calipers for intraoral use. No threshold values for anterior tooth movement were found. If a threshold existed, it would have been represented by force values considerably lower than those employed. Optimal rates of tooth movement were observed in groups of children where 50–75 gm. were applied. Increasing the magnitude of force did not produce any increase in the rate of tooth movement. A positive correlative was observed between force magnitude and pain and tooth mobility. The amount of total tooth movement during a 27-day period varied from 0 to 3.3 mm.

XI. CARIES–HUMAN. MARCH 19, FORENOON, SECTION A

121. Examiner Ability To Reproduce Findings of Carious Lesions in a Caries Control Experiment.—*Warren R. Hester and Norman O. Harris, Aero-Space Medical Center, USAF, Brooks AFB, Texas.* A study of examiner ability to reproduce clinical examinations for dental caries was carried out concurrent with an experiment in SnF_2 caries-prevention treatment. The technic used to evaluate size and tooth-surface location of carious lesions and restorations was that developed at Indiana University. Two dentists participated in the tests, re-examining either their own patients or each other's patients at selected intervals. Experimental variation as deter-

* This investigation was supported by Research Grant D-548 (C-2) from the N.I.D.R., U.S. Public Health Service.

mined after biometric evaluation of recorded information was approximately 4 per cent between the two examiners and 4 per cent between the findings of the same examiner when rechecking a given patient. Sources of experimental variation were determined to be (1) difficulty in reproducing estimation of doubtful lesions with both mirror and explorer and with X-rays; (2) difficulty in assigning definite location to lesions or restorations extending to or near the junctions of two or more tooth surfaces; and (3) administrative errors in calling by the examiner or in recording by the assistant. Errors in biometric integration and evaluation of data were negligible because of built-in cross-checks before and after IBM card print-out.

122. A Study of Beginning Enamel Caries.—*L. McMillan and L. S. Fosdick, Chemistry Department, Northwestern University Dental School, Chicago.* Freshly extracted teeth with beginning lesions were selected. After the tooth surface had been cleansed by means of a toothbrush, the surface of the lesion was blotted dry, and two types of replicas were taken. For observation under the electron microscope, polyvinyl alcohol–Form Var* replicas were taken and shadowed with chromium. In both cases the replicas were photographed. After the surface was examined, slices from the surface of the lesion 0.02–0.1 mm. thick were removed by means of a sharp file, and the surface thus produced was replicated and photographed in the same manner as before. This process was continued through the entire depth of the lesion, thus producing "serial sections" of the lesion. In all cases satisfactory initial replicas could be secured with Fax Film† for observation with the light microscope, but only those lesions with the slightest penetration could be satisfactorily replicated for observation with the electron microscope. However, after the first or second slice had been made, there was sufficient material in the interrod spaces to support the polyvinyl–Form Var replica, and from then on, to the depth of the cavity, satisfactory replicas could be secured. In general, the lesion progressed by removal of the inorganic substance from between the rods, leaving an amorphous mass. In no case were micro-organisms visible in the substance between the rods at a depth where satisfactory replicas could be made. The rod ends appeared intact, but uneven, probably because of fracture during the sectioning. Toward the depth of greatest penetration, inorganic crystals were visible in the interrod material.

123. A Comparison of Naturally and Artificially Produced Lesions in Enamel.—*E. Smith and M. B. Quigley, University of Alabama Medical Center, Birmingham.* Light microscopy and microradiographic technics have been used to study naturally and artificially produced demineralized zones in enamel, with special attention directed toward "white spots." Extracted teeth showing lesions were sectioned in one of three planes—longitudinally, coronally, and at right angles to the long axis of the enamel rods. Naturally occurring white spots showed subsurface demineralization, the extent of which was greater than observed by light microscopy. The demineralization as observed in the longitudinal and cross-sections followed the direction of the enamel rods in their course toward the amelodentinal junction. The cross-sections showed considerable lateral extension of the subsurface demineralization, and the spread could not be correlated with any histologic structure. One notable finding was that using the same exposure on the microradiographs but varying the developing time gave marked differences in the results. Where natural cavitation had occurred, there appeared to be a progressive loss of mineral as the amelodentinal junction was approached. Artificial demineralization was produced by using acidified gelatin (Mühlemann, *Helvetica Odont. Acta,* **3**:35, 1959). Microscopically and grossly, these lesions were identical with natural white spots. Immersion in the solution up to 15 days with constant stirring failed to produce cavitation.

* Shawinigan.

† Brush Development Co., Cleveland, Ohio.

124. The Effect of Phosphates on Acid Fermentation in Saliva.—*L. S. Fosdick and E. J. Krob, Chemistry Department, Northwestern University Dental School, Chicago.* Caries-active saliva-sugar mixtures were allowed to ferment in the presence and in the absence of varying amounts of added phosphates for a period of 7 hours. The rate of acid formation was determined by pH measurements at hourly intervals. The twenty-three phosphates tested in the above manner consisted of the sodium, potassium, or ammonium salts of a number of ortho-, meta- and pyrophosphoric acids. It was found that none of the orthophosphates inhibited acid formation. The meta- and pyrophosphates inhibited acid formation in a concentration of 100 mg/100 ml. Inhibition of acid formation was also produced by seven of the meta- or pyrophosphates in a concentration of 50 mg/100 ml of saliva.

125. Incidence of Dental Caries during and after Pregnancy.—*George S. Easton, College of Dentistry, State University of Iowa, Iowa City.* A comparison was made between caries activity in a group of 120 pregnant women and in a control group of a similar number of unmarried females of corresponding ages. The age range for both groups was seventeen to thirty-four years. Thorough clinical examinations were completed on three occasions, with one examiner conducting all examinations. A roentgenographic survey was included in the initial examination; bite-wing projections were obtained in subsequent examinations. Caries-prevalence data was obtained prior to the fifth month of pregnancy. A second examination was conducted within 3 weeks of the date of delivery. The final examination was completed approximately 12 months thereafter. Examinations of the patients comprising the control group followed a similar time schedule. Caries activity was expressed in the order of new and recurrent lesions found during the second and third examinations. For the second examination, the mean number of carious lesions was 0.755 per person for the experimental group and 0.730 for the control group. The mean number of lesions detected during the third examination was 2.088 per person for the experimental and 2.277 for the control group. For the period of the study, the mean increase per person in new and recurrent carious lesions was 2.842 for the experimental group. In the control group it was 2.957. In neither case was significance attached to the difference by the chi-square statistical test.

126. Clinical Trials of Caries-inhibitory Dentifrices.—*F. M. Keys, Dental Department, Great Lakes, Illinois, N. J. Overton and T. W. McKean, U.S. Naval Academy, Annapolis, Maryland.* A clinical study to determine the anticariogenic effect of toothpaste was conducted over a 2-year period at the U.S. Naval Academy, Annapolis, Maryland. Approximately 400 midshipmen used pastes containing stannous fluoride or sodium fluoride plus lauroyl sarcosinate, while a similar-sized control group used placebos. The study was conducted according to standard survey methods except that the actual counts were based on new carious lesions rather than the usual DMFS index. This was possible because all carious lesions, except very tiny interproximal etchings which did not penetrate the enamel, were restored at the beginning of the study. At the end of the first year there was no correlation between the amount of decay and the contents of the dentifrices. At the end of the second year the stannous fluoride users showed slightly fewer lesions, while the sodium fluoride–lauroyl sarcosinate users showed slightly more lesions than did the controls. When subjected to statistical analysis, no significant differences were found at the 5 per cent level. In fact, most of the differences noted were not even significant at the 10 per cent level of confidence. Therefore, in young men of Naval Academy age, these particular decay-inhibiting ingredients failed to provide the benefits which have previously been reported by others.

127. An in Vitro Method for Producing Carious Lesions.—*Marion D. Francis and A. H. Meckel, Miami Valley Laboratories, Procter & Gamble Co., Cincinnati.* A new in vitro method of producing tooth decalcification similar to carious lesions in man was devised. Decalcification was induced in 2–4 days in a glucose–tomato-juice

agar medium inoculated with saliva. The extent of decay or decalcification was quantitatively evaluated by fluorescence methods, transmitted light, staining reactions, and visual grading. The types of lesions obtained in vitro were compared with incipient and fully progressed carious lesions produced in vivo and were found to be indistinguishable. The caries-inhibiting effect of agents such as NaF, SnF_2 and $SnCl_2$, applied by brushing or by immersion, were tested both in the Pigman artificial mouth and in the agar-glucose-saliva system. It was found that the ranking of the inhibiting effect of these topical agents alone and in some paste combinations agreed well with the available data from animal and clinical tests.

128. A Device for Thin-Sectioning of Hard Tissues.—*John A. Gray and Donald L. Opdyke, Miami Valley Laboratories, Procter & Gamble Co., Cincinnati.* Thin-sectioning of hard tissues such as bones and teeth for light microscopy has not developed to the satisfactory degree as has that for soft tissues. For this reason, a machine was constructed which could routinely cut practically polished sections ready from samples of these hard tissues. Sections as thin as 10–40 μ in thickness were obtained, depending upon the nature of the hard tissue. Any adhering soft tissue was preserved, and soft tissue alone could be sectioned even thinner than the hard tissues. Basically, the machine consists of a circular saw blade held horizontally by a vertically mounted motor which is vertically adjustable in micron increments. The sample was clamped to a milling table providing accurate positioning in the horizontal plane. The table was powered to feed the sample automatically to the saw. Saw marks were minimum, obviating any grinding or polishing step, and a water mist or bath prevented overheating and drying during cutting. Sample loss during serial sectioning could be held to the blade thickness of 150 μ. Special sample preparation was required only for soft tissues. These sectioning results are illustrated with photomicrographs of teeth, bone, and adjacent tissues. A second similar device composed only of available machine-tool components is described for routine, but less precise, cutting of hard tissues.

129. The Histochemistry of Dental Caries.—*Donald L. Opdyke, Miami Valley Laboratories, Procter & Gamble Co., Cincinnati.* The application of histochemical methods to thin sawed sections of human carious teeth enabled us to make the following observations: (1) The carious lesion was histochemically the most reactive site in teeth. (2) Reactions for calcium were intensely positive in the carious lesion, including small "white spots," indicating that the enamel mineral component in this site was more loosely bound than in the sound tooth. (3) Tests for protein gave intense reactions at the same sites. An intense protein reaction was demonstrated in the entire section by gentle demineralization. In the carious spot the protein, as well as the mineral, was more loosely combined. (4) Refluxing the sections in ethylenediamine eliminated the protein reaction and extended the mineral reaction to the entire section. The pH of the carious lesion was reported and a description given of the lipid component of enamel rods, with histochemical evidence for its origin in enamel and spread into the dentin. By using the agar method of Francis and Meckel (see abstracts of papers presented at this meeting), it was possible to induce carious lesions in human teeth that were indistinguishable morphologically and histochemically from naturally occurring dental decay. In early dental decay the principal etiologic agent was acid attack. A time relationship was established for the order of appearance of the histochemical reactions in the decay process: first, unbound mineral; second, the acidic reaction; third, uncoupled protein; and fourth, unmasked lipids.

130. Visual and Chemical Effects of Surface Acid Penetration into Tooth Slices.—*L. W. Wachtel, U.S. Naval Dental School, National Naval Medical Center, Bethesda.* The effect of acids on enamel and dentin of human teeth was studied with the aid of specially prepared tooth slices. Sections of longitudinally sliced teeth, protected on the cut sides by cemented plastic, were exposed to weak acid solutions (0.01–0.07 N lactic acid and/or 0.01 N hydrofluoric acid) for periods of up to 1 year. Phos-

phorus analyses of the acid solutions indicated the degree of solubility of the exposed areas, while visual observation permitted determination of the extent of dissolution. Results indicated that the acids attacked the enamel radially from point of contact, with no selectivity noted for any areas of the enamel. In the dentin the lactic acid showed no tendency to follow the dentinal tubules. Exposure to methylene blue showed more uptake of the dye into some of the tubules, but the acid progressed evenly along the entire line of exposure. Although the rate of dissolution of the enamel as measured by phosphorus in solution was two to three times faster with hydrofluoric acid than with lactic acid, the area involved was less extensive. Lactic acid produced a whitening of the enamel but no visible erosion; the hydrofluoric acid produced erosion but no whitening. Exposure to lactic acid following hydrofluoric acid treatment showed a slower rate of dissolution for a short period of time.

131. THE pH IN CARIES AFTER GLUCOSE, BUFFER, OR EXCAVATION.—*Thomas R. Dirksen, M. F. Little, and B. G. Bibby, Eastman Dental Dispensary, Rochester, New York.* The amount of decayed material and the size of the clinical openings of 97 cavities were correlated with the initial pH and the response to glucose and phosphate buffer. Cavities were also excavated and the pH differences in the newly exposed surfaces reported. Using visual and X-ray measurements, cavities were classified into the following types: (I) small clinical openings and a thick layer of decay; (II) wide clinical openings and a thick layer of decay; and (III) wide clinical openings and a thin layer of decay. The three types of cavities had different initial pH values and responded differently to a 10 per cent glucose solution. The average pH value of all type I cavities was lowest both initially and during the 20-minute period after glucose. Type III cavities under similar conditions had the highest average pH values. After isolation, 0.1 M phosphate buffer (pH 6.8) was added to cavities. Type III cavities reached a pH of 6.8 in a shorter length of time than did types I and II. On attaining a pH of 6.8, the buffer was removed, and the time necessary for a return to the original pH was recorded. The length of time varied for all three types. The pH in the depths of newly excavated cavities was significantly lower than the corresponding surface layers.

132. ACID PRODUCTION AND ENAMEL DECALCIFICATION IN FOOD-SALIVA MIXTURES.—*Basil G. Bibby and Narendar N. Soni, Eastman Dental Dispensary, Rochester, New York.* Thirty-five foodstuffs representing flour, flour products, sugar products, milk products, desserts, cereals, and candies were incubated in saliva, and tooth sections, of which only a window was unprotected, were kept in each test mixture. Examination for decalcification was made under polarized light after 0, 6, 8, and 24 hours. pH measurements were made at the same times. Titrations were made at 24 hours to determine the amount of acid formed during fermentation. Poor correlations were found between the extent of decalcification and acid formation. Milk products and unrefined cereals produced most acid but showed little decalcification.

133. SALIVARY OXIDATION-REDUCTION RATES AND THEIR RELATION TO DENTAL CARIES ACTIVITY.—*George H. Green, Dental Research Facility, U.S. Naval Training Center, Great Lakes, Illinois.* Since previous manometric studies in this laboratory showed that saliva from caries-rampant subjects had a significantly higher substrate oxidation rate than did saliva from caries-immune individuals, the present study was undertaken to determine whether there was also a significant difference in their total O/R patterns. Thousands of naval recruits were screened, and 76—32 with extremely rampant decay and 44 having complete immunity—were chosen to represent the extremes of dental caries activity. Individual salivary samples were combined with a buffered substrate solution and their O/R potentials followed potentiometrically. The caries-rampant group exhibited a precipitous fall in potential, dropping almost 400 mv. in the first 20 minutes. During the same period the caries-immune group dropped only 100 mv. These differences were statistically significant and indicated that the salivary O/R rate would be a useful test for distinguishing the presence or absence of dental

caries activity. In search of a more practical means of determining these rates, tests were run with various O/R indicator dyes. The reduction of resazurin to its first reduction product, resorufin, was the only one with which it was found possible to duplicate the potentiometric findings. The resazurin did not alter the normal O/R patterns of the saliva, was irreversibly reduced at a suitable E_h range, and presented a very distinctive color change in its reduced form. It was concluded that the resazurin reduction time of saliva shows much promise as a simple and practical test for dental caries activity.

XII. HISTOCHEMISTRY, CYTOLOGY, AND CARCINOGENESIS. MARCH 19, FORENOON, SECTION B

134. Enzyme Histochemistry of the Tooth Germ.—*Giuliano Quintarelli, School of Dentistry, University of Alabama Medical Center, Birmingham.* The distribution of hydrolytic and other enzyme systems was studied in tooth germs of rats from embryos at 18 and 20 days and in newborn rats at 2, 3, and 4 days. The hydrolytic enzymes surveyed included acid phosphatase, total non-specific esterases, beta-D-galactosidase, and others. Fixed-frozen sections were employed in the study of the above-mentioned enzymes. Succinic dehydrogenase and aminopeptidase were studied in fresh-frozen sections. The substrates employed were 6-benzoyl-2-naphthyl-phosphate for acid phosphatase; 6-bromo-2-naphthyl-beta-D-galactopyranoside for beta-galactosidase; naphthol-AS acetate for total non-specific esterases. The succinic dehydrogenase system was investigated by means of the Rosa and Velardo modification of the Seligman and Rutenburg technic. The beta-glucuronidase was detected by the method of Fishman and Baker, which employs 8-hydroxyquinoline glucosiduronic acid as substrate. The enzymes were found in the same general areas. The odontoblastic and ameloblastic layers, the stratum intermedium, and the alveolar bone showed reactivity with differences in intensity according to the type of enzyme.

135. Histochemical Studies on Wound Healing in Experimental Lathyrism.—*George A. Krikos, University of Rochester, Rochester, New York, and University of Pennsylvania, Philadelphia, and J. Lowell Orbison, University of Rochester.* These studies were conducted for the purpose of obtaining knowledge about the nature of the connective tissue change in experimental lathyrism. Weanling and adult male rats were divided into groups of eight rats each. All animals were pair-fed and received Purina Fox Chow; 0.4 per cent beta-aminopropionitrile fumarate was added to the diet of the experimental rats. On the ninth experimental day an excision wound was made into the chest wall. Control and experimental groups of weanling rats were sacrificed on the third and ninth days after wounding, while similar groups of adult rats were sacrificed on the third and twelfth days. The wounds with their surrounding tissues were excised, fixed in formalin, and stained with hematoxylin and eosin, silver stain, Van Gieson method, P.A.S. with and without previous amylase and hyaluronidase digestion and pyridine extraction, Hale's iron adsorption technic with and without previous hyaluronidase digestion, and toluidine blue (pH 2 through 7) before and after sulfation and with and without previous hyaluronidase digestion. Comparison of the wounds of the control and experimental animals revealed only quantitative histochemical differences. The 9- and 12-day-old experimental wounds were characterized by the persistence of numerous amorphous areas staining positively with P.A.S. and sulfation metachromasia and containing numerous fine, disoriented fibers exhibiting staining characteristics consistent with collagen but without formation of bundles. The P.A.S. stainability was not affected by previous amylase and hyaluronidase digestion or pyridine extraction, and the sulfation metachromasia was not affected by previous hyaluronidase digestion. The inability to form bundles of fibers may have been due to an abnormality in the collagen protein, in the ground substance polysaccharides, in other environmental factors, or in a combination of these.

136. QUANTITATIVE MICROHISTOCHEMICAL STUDIES OF ALKALINE PHOSPHATASE IN HAMSTER TOOTH BUDS.—*Klaus Nuki, Division of Oral Pathology, University of Illinois College of Dentistry, Chicago, and Sjoerd L. Bonting, Department of Medicine, Presbyterian–St. Lukes Hospital, Chicago, and Department of Biological Chemistry, College of Medicine, University of Illinois, Chicago.* First maxillary molar tooth buds were frozen in liquid nitrogen, sectioned at 16 μ in a cryostat, and frozen-dried. The sections were divided into ten topographically distinct zones by microdissection, and the fragments were incubated with a suitable substrate. Enzyme activity was determined by spectrophotometric methods. Alkaline phosphatase activity ranged from 398 moles of substrate split per hour per kilo dry weight (MKH) in the stratum intermedium 4 days after birth, to 0.44 MKH in the oral epithelium just before birth. There was an increase in activity with age in most zones, with the exception of the ameloblasts and enamel plus dentin, where activity fell. (Ameloblasts 60 MKH just before birth, 1.3 MKH at 4 days; enamel plus dentin 16 MKH, 0.59 MKH at 4 days.) The subodontoblastic layer of the dental papilla was half as active (211 MKH) and the odontoblasts about a third as active (144 MKH) as the stratum intermedium. The activity of the dental papilla fell with distance from the calcifying areas, that portion next to the cervical loop showing extremely low activity (12.3 MKH at 4 days). The two zones showing highest activity—the stratum intermedium and the subodontoblastic papilla—were not adjacent to the calcifying enamel and dentin. The stratum intermedium was found to have an activity thirteen times as high as that found in the proximal tubules of the kidney. Results were also compared with those obtained by conventional histochemical staining methods.

137. COMPARISON OF ALKALINE PHOSPHATASE ACTIVITY IN ORAL TISSUES WITH DIFFERENT HISTOCHEMICAL TECHNICS.—*M. S. Burstone, National Institute of Dental Research, National Institutes of Health, Bethesda.* This work relates to localization of alkaline phosphatase in oral tissues employing diverse histochemical procedures. All tissues employed in the present work were frozen-dried and imbedded in paraffin. They consisted of the jaws and adjacent oral epithelium of immature (1–10-day-old) mice, hamsters, and rats. In addition, twenty specimens of human gingiva were utilized. Both glycerophosphate and azo dye technics were compared. The substrates employed in the simultaneous coupling azo dye method were phosphates of 2-hydroxy-3-naphthoic acid anilides. Twenty-two such compounds were employed. A new post-coupling procedure, employing the phosphates of 5,6,7,8-B-tetralol-carboxylic acid-B-naphylamide or 2-hydroxy-3-naphthoic acid-2-aminoanthraquinonylamide were also used. Since the former naphthol is fluorescent, it was possible to visualize sites of activity under ultraviolet light without coupling with a diazonium salt. This procedure represents a new principle in enzyme histochemistry. In general, the results were similar with all technics, with the exception that nuclei were frequently stained with the glycerophosphate method. This nuclear staining was most intense adjacent to sites of high activity (e.g., odontoblasts and subjacent pulp), thus suggesting a diffusion artifact. Staining of calcifying bone and dentin matrices with the glycerophosphate method is also considered to be artifactual (Gomori, *J. Lab. Clin. Med.*, **35**:802, 1950). Osteoblasts, osteocytes, and active perichondrocytes were highly active. In the developing molar teeth, characteristic activity was also seen in the stellate reticulum, stratum intermedium, and odontoblastic zones. Gingiva revealed strong activity of the capillary endothelium and processes of some fibroblasts. Since both simultaneous, post-coupling, and fluorescence methods give similar localizations, it would appear that the introduction of a diazo compound into the substrate mixture does not usually alter the specificity of the reaction.

138. MORPHOLOGICAL AND ELECTROCHEMICAL STUDIES OF A MUCOSAL SURFACE IN THE ORAL CAVITY.—*Milton B. Engel, Norman R. Joseph, and Lloyd Erickson, University of Illinois Professional Colleges and University of British Columbia.* Correlated studies of the colloidal properties and morphology of the cornified surface of the

gingiva were made in 6 female *Macaca mulatta*. Three animals were immature, and 3 were adults whose ovulatory cycle was known. The charge density of the surface colloids was measured at a series of pH's between 2.2 and 7.4. This yielded an in vivo titration curve of the heterogeneous proteins of the stratum corneum. At physiologic pH the surface was negatively charged. The charge decreased to zero or became positive as the pH was lowered. Menstruating adults and immature animals gave similar curves, characterized by maximum titrable groups below pH 3.7 and an isoelectric point around 2.1. In immature animals injected with estrogen or in adults at mid-cycle, the isoelectric point was elevated by about 0.5 pH unit, and the total number of titrable groups was increased. Dye binding at the gingival surface was also studied electrochemically, using an anionic dye* and a cationic dye.† Morphological studies were done on sections of frozen-dried tissues stained with hematoxylin and eosin. Also, the binding of the dyes was studied under controlled conditions of pH, temperature, and ionic strength by means of a microphotometer. Binding of the cationic dye was maximal at high pH (7.4) and diminished as the pH dropped. Binding of the anionic dye was maximal at low pH (2.2) and diminished as the pH rose. These results were attributed to electrostatic and short-range forces inherent in the colloid of the gingival surface. Preliminary attempts to identify some of the specific chemical substances or groups involved was discussed.

139. Succinic Dehydrogenase in the Enamel Organ of the Rat's Incisor.—*Edward J. Reith and William Zussman, Department of Anatomy, College of Medicine, New York University.* Incisor teeth with adherent enamel organs were removed from the jaws of rats and placed in a variety of media for the demonstration of succinic dehydrogenase. After incubation for 30 minutes at 37° C., they were washed in 1 per cent acetic acid and then fixed in 10 per cent neutral buffered formalin for 2 hours. The teeth and enamel organs were next imbedded in gelatin. The enamel organs were then shaved from the teeth and reimbedded in gelatin. Sections were cut in a cryostat and mounted in glycerogel. Attention was directed to that part of the enamel organ in which pigment was present in noticeable amounts only in the ameloblasts. The region was further characterized by the failure of pigment to extend into the distal extremity of the cells. Succinic dehydrogenase was detected by the reduction of blue tetrazolium in the presence of phenazine methosulfate. The reaction was significantly inhibited by the addition of malonate or iodoacetate. Omission of succinate resulted in a weak positive to negative reaction which was always weaker than the weakest reaction obtained when succinate was added. Succinic dehydrogenase was found in the epithelial ridges and stratum intermedium but not in the ameloblasts.

140. Use of the Papanicolaou Technic in Diagnosis of Oral Cancer.—*Richard W. Tiecke, Joseph C. Calandra, and Francis J. Kendrick, Northwestern University Dental School, Chicago.* Scrapings from the oral tissues of 150 persons were used in this study. Fifty persons had lesions clinically diagnosed as squamous-cell carcinomas. In these cases scrapings were taken from the surface of the lesion and from bordering areas, and the lesion was biopsied. One hundred persons entirely free of clinically apparent oral lesions were selected as controls. Scrapings were taken from the palate, buccal mucosa, gingiva, floor of mouth, and tongue. A clinical history was recorded in every case. The scrapings were spread on slides and were stained and interpreted by the Papanicolaou method. There were no false positives in the 100 control cases. In the 50 carcinoma cases there were seven false negatives, all early in the series.

141. A Study of Exfoliative Cytology by Papanicolaou Stain Technic.—*Marvin Jacobson, University of Illinois College of Dentistry, Chicago.* A detailed survey was made of the exfoliative gingival cells by taking individual smears of mar-

* Orange G, 5×10^{-4} M, The Harleco Manufacturing Co.

† Safranin O, 5×10^{-4} M.

ginal and papillary gingiva. This was done at various time intervals ranging from 1 day to 1 week. It was observed that smears varied greatly from normal to pathologic and that this method could be used for ascertaining the rate of keratinization in various clinical studies. The cells were classified into the following six categories: (1) totally keratinized (no nuclei); (2) outer superficial (pyknotic nuclei); (3) inner superficial (larger nuclei having membrane with visible structures and a large amount of cytoplasm); (4) intermediate (larger nuclei, smaller amount of cytoplasm); (5) outer basal (small amount of cytoplasm with large round or oval nucleus); and (6) basal (small cells with large round nucleus and small amount of cytoplasm). Photographs were taken of the smears to show the changes that had occurred. This method appeared to have some merit for the diagnosis of periodontal conditions.

142. Intraoral Cytology and Radiation Response (RR).—*Sol Silverman, Jr., University of California, San Francisco.* Previous work with vaginal cytologic smears in cervical carcinomas by Graham indicated a positive correlation between radiation response and 5-year cure rates. Her criteria were based on morphologic cell changes; a good response was indicated when the number of altered normal cells exceeded 70 per cent. A comparative cytologic evaluation of irradiation effects on intraoral carcinomas was attempted, with the aim that this information might supplement the present limited methods of evaluation. The contraindication for serial biopsy and the difficulty of obtaining representative sections are obvious. Smears from the malignant area and adjacent normal-appearing mucosa were obtained before radiation, each day during treatment, and on posttherapy follow-up visits. Morphologic and staining characteristics utilizing the modified Papanicolaou technic were noted and charted. Nonmalignant squamous cells from the lesion and adjacent mucosal surfaces reflected significantly the same responses. Those recorded included nuclear-cytoplasmic enlargement, nuclear clearing, multinucleation, and vacuolization. Measurment of changes per unit number of cells was referred to as "radiation response" (RR). Our work indicated a relationship between an increase in these cellular changes and the amount of radiation. However, the pathologic significance can be determined only by long-term follow-up statistical studies. There was no apparent correlation between the number or characteristic changes in malignant cells. Disappearance or persistence of malignant cells near the end of therapy has so far seemed to reflect tumor response.

143. Changes Associated with Keratinization in Lesions of Human Buccal Mucosa.—*Stanley J. Gerson, J. Meyer, and J. P. Weinmann, University of Illinois College of Dentistry, Chicago.* The purpose of this investigation was to observe qualitative and quantitative cellular changes associated with the transformation of an unkeratinized into a keratinized epithelium. A type of lesion was sought that would show, on the same section, areas of both unkeratinized and fully keratinized epithelium. These requirements were fulfilled by biopsy specimens of fibroma or fibrous hyperplasia of buccal mucosa. By virtue of its location, the epithelium at the apex of such a lesion is subjected to mechanical irritation by the teeth and, as a result, transforms into a parakeratotic or keratinized epithelium. The epithelium at the base of such lesions usually remains unaffected and may then be used to study the point of departure. The present study included only the specimens containing fully keratinized regions and a region of normal unkeratinized epithelium for comparison. The specimens were stained with a variety of stains permitting comparison or morphologic detail and of some chemical components of the cells. In addition, the total thickness, the average cell size, and the mitotic index were derived and compared. Keratinization led to changes in the following traits: thickness, cell size, mitotic index, staining of cytoplasm and cell membranes, concentration of glycogen, occurrence of tonofibrils, intercellular bridges, and keratohyalin granules.

144. Effects of Topical Application of Cortisone Acetate on Carcinogenesis in Hamster Pouch.—*W. R. Sabes, A. P. Chaudhry, and R. J. Gorlin, University of Minnesota School of Dentistry, Minneapolis.* Thirty-five golden Syrian male ham-

sters, approximately 40 days old, were divided into three groups of 10, 10, and 15 animals each, respectively. The animals of Group I were treated by painting the right cheek pouch with 0.05 cc. of 0.5 per cent 7–12 dimethyl benz (alpha)anthracene in liquid petrolatum. The left pouches were treated in the same manner with liquid petrolatum alone. Group II was treated by swabbing the right buccal mucosa with 0.05 cc. of the carcinogen, allowing this to dry by everting the pouch for 3 minutes, and then painting the same area with 0.05 cc. of 0.025 per cent cortisone acetate in normal saline. Animals of Group III were similarly treated, except that the application of cortisone acetate preceded the application of the carcinogen. No control for cortisone acetate was employed, since its non-carcinogenic nature has been previously established. The paintings were repeated twice weekly for a total of 19 applications over a 67-day period. The latent period for Group III (cortisone first) was 46 days; for Group I (carcinogen control), 53 days; and for Group II (cortisone after), 60 days. The average number of clinical papillomas affected in each animal was 1.50, 1.83, and 3.08 lesions in Groups I, II, and III, respectively. At any stated time during the study there was a significant difference in the incidence of induced lesions in Group III over the control (Group I). Histologic studies are now being done to ascertain the qualitative differences between the lesions in the various groups.

145. Rhythms in Blood Eosinophils and Mitoses of Hamster Pinna and Pouch; Phase Alterations by Carcinogen.—*A. P. Chaudhry and F. Halberg, Schools of Dentistry and Medicine, University of Minnesota, Minneapolis.* An organism's physiologic rhythms can be made to assume periods corresponding to certain environmental periodicity by exposure, e.g., 12-hour periods of alternating light and darkness. Light-dark, synchronized-periodicity analysis resolves rhythms in this frequency-synchronized state and describes reproducible phase relations among physiologic variables. Phase differences among functions thus come to the fore and may be plus, minus, or zero (Halberg *et al., Photoperiodism and Related Phenomena in Plants and Animals,* AAAS Pub. No. 55 [1959]). Maps in time (from light-synchronized-periodicity analysis) bearing on cell division and underlying phenomena provide a normal temporal reference standard against which alterations prior to (or after) the appearance of cancer might be evaluated. Such maps in time were obtained at 4-hour intervals for 24-hour periods on Golden hamsters for cell division in pinna and pouch epithelium and for blood eosinophils. These three rhythms showed peaks during the daily light period. Thereafter, alterations of mitotic phase relations were recorded in hamsters injected intrasubmandibularly with 0.05 cc. of a 0.5 per cent solution of 7:12 dimethylbenz(α)anthracene in liquid petrolatum. These results, underlying factors, and the methodology of periodicity analysis as it applied to the exploration of precancerous physiologic changes were discussed.

XIII. E. H. HATTON AWARD COMPETITION II. MARCH 20, FORENOON, SECTION C.

146. Effect of Molybdenum on Fluoride Absorption.—*Daniel B. Crane, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* Previous studies in these laboratories have shown that the presence of molybdenum is associated with a significantly greater skeletal retention of fluoride than takes place in its absence. In order to learn more concerning the mechanism by which this occurs, studies were designed to determine whether molybdenum affects the absorption of fluoride. Adult rats were anesthetized with sodium nembutal, and the stomachs were completely ligated. Fluoride and molybdenum were injected into the ligated stomach, and after 30 minutes the stomach was removed, its contents prepared for fluoride analysis, transferred to a fluoride distillation flask, and the fluoride remaining in the stomach used as an indication of fluoride absorption. Such studies, performed under various experimental conditions on both the stomach and the small intestine, showed that molybdenum increased fluoride absorption in the intestine but not in the stomach.

147. FLUORIDE AVAILABILITY FROM FLUORIDE TABLETS.—*Donald E. Derrow, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* Previous studies conducted at Indiana University have shown that a single dose of sodium fluoride containing 1.0 mg. F in tablet form was rapidly cleared by the plasma and excreted in the urine. Under such physiologic conditions it seemed impossible to maintain those conditions in the blood which must be present in order to have optimum substitution of fluoride in the developing tooth necessary for producing resistance to dental caries, as is known to occur when a child consumes drinking water and food prepared from this water at a level of 1 μg F ml many times throughout the day. In order to learn more about the site of fluoride absorption in the body and the effect of fluoride availability as a function of pH, a commercially available fluoride tablet containing 1.0 mg.F was studied to learn the amount of fluoride released in vitro at different pH values and as a function of time. The data showed that more fluoride was available at lower pH values. At a pH 2.0, about 95 per cent of the available fluoride was released during the first hour. Human studies with the fluoride tablet similarly indicated a rapid clearance of the fluoride, thus suggesting that a single dose of fluoride as furnished by a fluoride tablet will not duplicate the biological situation obtained from ingesting an optimum fluoride water supply several times throughout the day and eating food prepared from such water.

148. NERVE REGENERATION IN REPLANTED HAMSTER TEETH.—*W. Roger Sorg, University of Pennsylvania School of Dentistry, Philadelphia.* Mandibular second molars of 10 Syrian hamster littermates, 18–32 days old, of both sexes were extracted and immediately replanted under parenteral nembutal. Animals were sacrificed between 34 and 63 days of age, yielding replants for histologic study which had been maintained in place for 16–43 days. At sacrifice, 16 of the 20 replanted teeth were in position. The mandibles were fixed in 10 per cent formalin, decalcified, paraffin-imbedded, serially sectioned, and stained with hematoxylin and eosin and modified Power's silver stain. Examination showed root resorption in 11 of the retained teeth, while cementum deposition and periodontal membrane reattachment were observed in all 16 replants. Nerve regeneration was observed in 9 of the 16 replants, with centrally located nerve trunks ramifying into beaded fibrils which terminated in free or beaded endings within the odontoblastic area. Some fibrils ended within the predentin of the cuspal areas, but most nerve tissue terminated at, or within, the odontoblastic layer. Nerve regeneration occurred in those teeth which contained histologically normal pulp tissue. Odontoblastic production of primary, secondary, and osteodentin occurred in all replants containing regenerated nerves. A correlation could be established between the amount of neural regeneration and the nature of dentin production following tooth replantation. This study also demonstrated that peripheral nerve regeneration may take place in the canals and pulp chambers of replanted hamster teeth.

149. ACID PRODUCTION AND ENAMEL DECALCIFICATION BY FERMENTING FOODSTUFFS.—*R. J. Andlaw, Eastman Dental Dispensary, Rochester, New York.* The purpose of this study was to compare the amount of acid produced by incubating different foodstuffs in saliva and to relate this to the amount of enamel decalcified by this acid. Blocks of enamel weighing 75 $\pm$ 5 mg. were taken from central incisor bovine teeth and suspended in the food-saliva mixtures. After 6 hours at 37.5° C., the enamel weight loss was determined, and the acid formed was measured by titration with sodium hydroxide. From 28 foods tested, it was shown that there was no direct relationship between the amount of acid produced and the amount of enamel decalcified. A coconut-filled chocolate bar produced less acid than milk chocolate but five times as much enamel weight loss. White flour produced less acid but more decalcification than the less refined whole-wheat flour, and, similarly, white bread produced less acid but more decalcification than whole-wheat bread. Among the breakfast cereals, bran formed the most acid but caused the least enamel weight loss, while corn produced the least acid but the greatest weight loss. Wheat germ, while forming an unusually large

quantity of acid, caused a relatively small enamel weight loss. In general, the results suggest the presence in some foods of factors capable of protecting enamel against acid decalcification. In unrefined flour and its products, the protective factors appeared to be associated with the bran and germ fractions, which are removed in the refining process.

150. Basal Metabolic Rates of Salivariadenectomized, Salivary-Duct-Ligated, and Control Rats.—*Ronald Bowman, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* Recent studies have suggested that rat salivary tissue has an accessory endocrine function, although the evidence is conflicting. This investigation was designed to provide additional information concerning such a relationship and to extend it to include the effect on respiration of animals with different levels of thyroid function. Three groups of animals were subjected to a laboratory-tested respiration chamber for BMR computation. Group I contained non-operated controls, Group II contained surgically salivariadenectomized rats, and Group III consisted of complete salivary duct ligation. Two series of studies were performed, the first using animals ingesting food ad libitum and the second using animals that were pair-fed. The BMR of salivariadenectomized and ligated animals was found to be significantly lower than that of control animals with intact salivary glands. Ligated animals demonstrated the greatest reduction in respiration, suggesting that the biological effects of duct ligation were different than those caused by surgical removal of the gland.

151. The Intermediary Metabolism of Rat Mucosa.—*Jules M. Snitzer, Saint Louis University.* The tissue covering the hard and soft palate was dissected out of 4–5-month-old Saint Louis University strain white male rats, and the intermediary metabolism, as indicated by oxygen uptake, was studied. The direct method of Warburg was used, and readings were taken at hourly intervals for 5 hours. Antibiotics were added to inhibit bacterial proliferation. The effects of the following Embden-Meyerhoff substrates upon the oxygen uptake of this tissue were examined: glucose, mannose, fructose, glucose-1-phosphate, fructose-6-phosphate, fructose-1-6-diphosphate, lactic acid, and pyruvic acid. The substrates examined in the Krebs cycle were acetate, citrate, isocitrate, alpha-ketoglutarate, succinate, fumarate, malate, and oxaloacetate. The oxygen uptake per milligram wet weight per hour was calculated and statistically compared with endogenous controls. Any differences below the 5 per cent level of confidence were considered to be significant. Fructose and fructose-1-6-diphosphate were the only Embden-Meyerhoff substrates capable of stimulating respiration during the experimental interval. The Krebs cycle substrates which stimulated respiration during the same period were acetate, succinate, fumarate, and oxaloacetate. The utilization of fructose and fructose-1-6-diphosphate suggests that many of the Embden-Meyerhoff scheme enzymes were present within the mucosa. The failure of the remaining substrates to stimulate respiration might have been due to their inability to diffuse into the cell. The same reason might have accounted for the failure of several of the Krebs cycle substrates to stimulate respiration. However, four of the six substrates present in the dicarboxylic acid cycle were metabolized, and this suggests that this cycle, rather than the tricarboxylic cycle, may have been operative in this tissue.

152. Thromboplastic Activity of Human Saliva.—*Hristo C. Doku, Department of Oral Surgery, Tufts University School of Dental Medicine, Boston.* Previous studies have indicated that saliva decreases the clotting time of whole blood. The purpose of this study was to determine what factors present in saliva were associated with the acceleration of the blood-clotting mechanism. Whole, parotid, and submaxillary saliva from 30 subjects was used to replace the commercial tissue thromboplastin in the prothrombin time determination by the one-stage modified Quick method. The partial thromboplastin test involved whole saliva, supernatant and sediment therefrom, and

submaxillary saliva from 20 subjects. Each of these liquids was substituted for cholesterol brain extract (CBE). The thromboplastin generation test was performed by replacing serum and/or adsorbed plasma and/or cholesterol brain extract with whole (mixed, supernatant, and sediment), parotid, submaxillary, and sublingual saliva. The tests were carried out on 30 subjects. It was found that mixed saliva and the pure parotid, submaxillary, and sublingual secretions had thromboplastic activity similar to that found for tissue and plasma thromboplastin. This activity varied among individuals and was dependent on the source of the secretion; the submaxillary secretion was more active than the mixed, sublingual, or parotid liquids.

153. The Effect of Stannous Fluoride on Human Gingival Tissue.—*Robert P. Swieterman, Indiana University School of Dentistry, Indianapolis.* During the routine use of high concentrations of stannous fluoride for topical applications in adults, it has been observed that when a gingival irritation is present, some tissue reaction results from the high concentration of stannous ions used. This investigation was designed to determine what effect stannous fluoride has on gingival tissue when topically applied. Normal and periodontally involved tissues were obtained from patients at Indiana University. The tissue to be removed was photographed and given a topical application with either stannous or sodium fluoride, stannous choloride, or sodium choloride solutions and was immediately rephotographed. Treated areas were surgically removed by gingivectomy immediately, or at 1-, 2-, 7-, 14-, 21-, and 28-day intervals. The tissue was placed in 10 per cent formalin and stained with hematoxylin and eosin. Histologic studies included an evaluation of the degree of inflammation, types of inflammatory cells present, depth of penetration into the epithelium, and length of time that the coating remained in the tissues. The results of this study showed no effect upon the gingival tissue either histologically or clinically from sodium chloride or sodium fluoride. When stannous fluoride or stannous chloride was used, a white coating, which will be referred to as a "local tissue precipitate," was noted. This local tissue precipitate disappeared clinically after the first day. As the degree of severity of the gingival inflammation increased, so did the degree of tissue precipitation. With "normal" gingival tissue no, or, at most, only a moderate, tissue precipitation of the gingival margin was observed. No change in amount or type of inflammatory cells was found after treatment with any of the agents. The depth of penetration of both tin salts was observed to be in the keratin layer and in the upper portion of the spinous-cell layer, depending upon the amount of inflammation present. One may conclude from these studies that, clinically, no injurious reaction results from stannous fluoride on gingival tissues even when chronic inflammation is present.

154. Study of Tooth Storage.—*Richard Coburn, Tufts University School of Dental Medicine, Boston.* The immediate consideration was the value of freezing as a method of storage and preservation for hamster teeth, to be later transplanted. Sixteen lower third molars of 32-day-old male hamsters were extracted and stored at −20° C. The teeth were placed in isotonic saline with benzylconium chloride and tetracycline hydrochloride and frozen. Autogenous transplants were carried out on the first, second, fourth, and eighth days following extraction. The teeth were replaced in the freshly prepared second molar sockets on the same side from which they had been removed. The animals were sacrificed on the ninetieth postextraction day, and the jaws were placed in formalin for later histologic examination. The results of the clinical examination showed four out of four good-to-excellent transplants in those carried out on day 1 and two out of eight on the fourth and eighth postextraction days. Poor results on the second day (0 out of 4) were attributed to accidental thawing and refreezing, suggesting the need for continuous refrigeration. The criteria for evaluating these transplants were absence of inflammation, degree of mobility or firmness, color of the transplant, arch position and relation to the occlusal plane, and radiographic examination.

155. Pilocarpine-stimulated Saliva from Fistulated Dogs.*—*S. S. Rothman, Department of Physiology, Schools of Dentistry and Medicine, University of Pennsylvania, Philadelphia.* Submaxillary and parotid saliva was collected separately under oil from two dogs with chronic fistulae and from a third dog with a submaxillary fistula only. All dogs weighed 12 kg. Pilocarpine, 0.33 mg/kg, was injected intravenously in a single dose. The volume and concentrations of Na^+, Ca^{++}, K^+, HCO_3^-, and Cl^- of each 15-minute specimen were determined 15–90 minutes after the pilocarpine injection. The volume of submaxillary saliva decreased from 18.7 ml. $\pm$ 0.5 S.E. in the first 15 minutes to 4.6 $\pm$ 0.6 in the last. Parotid saliva fell from 8.6 ml. $\pm$ 0.03 to 2.1 $\pm$ 0.3. For submaxillary saliva the concentrations of ions changed over 90 minutes as follows: Na^+, 104 mEq/l $\pm$ 4 to 70 $\pm$ 7; K^+, 13.7 $\pm$ 0.8 to 8.3 $\pm$ 0.7; Ca^{++}, 4.4 $\pm$ 0.3 to 3.7 $\pm$ 0.2; Cl^-, 88 $\pm$ 2 to 56 $\pm$ 5; and HCO_3^-, 26 $\pm$ 1 to 18 $\pm$ 2. The parotid saliva concentrations fell as follows: Na^+, 124 $\pm$ 8 to 89 $\pm$ 24; K^+, 11.9 $\pm$ 0.5 to 8.4 $\pm$ 1.1; Ca^{++}, 8.5 $\pm$ 0.5 to 6.0 $\pm$ 0.8; Cl^-, 88 $\pm$ 6 to 67 $\pm$ 14; and HCO_3^-, 45.6 $\pm$ 3.3 to 22.0 $\pm$ 1.8. In a series of five experiments, 5–10 ml. of a 10 per cent solution of calcium gluconate were given intravenously 45 minutes after the pilocarpine. Serum calcium determined 15 minutes after the gluconate injection showed a mean significant increase of 19 $\pm$ 1.2 per cent over control levels. The $(Ca)^{++}$ concentration in submaxillary saliva increased 77 $\pm$ 20 per cent over control levels, and the output of calcium increased 158 $\pm$ 23 per cent.

156. Microscopic and Radiographic Study of Oral Lesions in Dogs with Renal Osteodystrophy.—*Arthur B. Hattler, Department of Periodontology, Graduate School of Medicine, University of Pennsylvania, Philadelphia.* Severe chronic nephritis in dogs often produces marked osteodystrophic changes in the skeleton. The changes in the skeleton are minimum in comparison with those seen in the skull and particularly the jaws. The teeth become loose, and the jaws so compressible that the veterinary clinicians have come to call this condition the "rubber-jaw syndrome." It seemed of interest to study this condition with the purposes of (1) comparing the jaw lesions with the lesions in other bones, (2) studying the relationship of the epithelial attachment to the resorptive process in the alveolar process, (3) studying the changes in the alveolar bone proper (lamina dura), and (4) studying the changes in the supporting bone of the teeth. Eighteen dogs having renal osteodystrophy were disarticulated, radiographed, and the bones decalcified, sectioned, stained, and studied microscopically. Radiographically, osteoporotic changes could be discerned only in the skull. The jaw lesion consisted of a complete replacement of the alveolar process by fibrous connective tissue and immature bone. The periodontal ligament showed complete disorganization. In spite of these severe alterations, the epithelial attachment remained at the cementoenamel junction in all animals studied, even when calculus was present about the teeth. With complete resorption of the attachment apparatus and local irritating factors, pocket formation did not take place.

157. The Dental-Pulp Hemogram—Diagnostic Aid in Vital-Pulp Therapy.—*T. J. Guthrie, Indiana University School of Dentistry, Indianapolis.* It has been reported that the dental-pulp hemogram utilizing the first drop of blood from an exposed pulp can be used as a diagnostic aid in the selection of teeth for vital-pulp therapy. White-blood-cell differential counts (dental-pulp hemograms) were made from the blood of 53 cariously exposed deciduous and young permanent teeth and from 14 unexposed normal pulps. These were compared with the patients' peripheral hemogram. The teeth were evaluated as to history of pain, response to the electric pulp test, heat and cold, percussion, abnormal mobility, and the amount of hemorrhage from the exposure site. After obtaining the blood for the hemogram, the teeth were extracted and histologic sections prepared. An attempt was made to correlate the clinical, histologic, and hematologic findings. The teeth were classified as "good candi-

* This investigation was supported by Research Grant Py-103-2 from the N.I.H., U.S. Public Health Service.

dates" and "poor candidates," depending on the extent of pulpal inflammation. Hemograms with high lymphocyte counts and normal counts were found in both the "good" and the "poor candidate" groups. However, high neutrophil counts were found only in the "poor candidate" group, and often these cells showed signs of degeneration and karyolysis. Normal pulps exhibited hemograms with both high lymphocyte counts and normal counts. A history of pain and profuse bleeding occurred more often in the "poor candidate" group. Internal resorption was found only in the "poor candidate" group. The various testing procedures were found to be inadequate in determining the extent of pulpal inflammation.

XIV. CALCULUS FORMATION AND TISSUE REACTIONS TO DENTAL MEDICAMENTS AND CEMENTS. MARCH 19, AFTERNOON, SECTION A

158. MORPHOLOGY OF DENTAL CALCULUS.—*Jorgen Theilade and Helmut A. Zander, Eastman Dental Dispensary, Rochester, New York.* To study the morphology of dental calculus, 28 pieces of supragingival and 14 pieces of subgingival calculus were removed from the lower anterior teeth of ten patients. The organic material of the specimens was extracted with ethylenediamin. Then the specimens were rinsed in water, dehydrated in ascending alcohols, and infiltrated under vacuum with ascending concentrations of uncatalyzed plastic* diluted with styrene. Finally, the specimens were placed in catalyzed plastic and cured at 60° C. Serial sections of 40–70 μ were cut and the sections studied microscopically with transillumination, phase contrast, and polarized light. All specimens showed a granular appearance, and certain structures were seen with various frequency: (1) brushlike structures consisting of parallel threads arranged perpendicularly to a more or less curved line, several of these structures often being arranged in parallel bands resembling incremental lines; (2) tuft-like structures of more or less curved threads packed close together; (3) filamentous figures, often branching, frequently found; (4) dark matted areas found in most specimens. (5) occasional radiating figures resembling "crystal formations"; and (6) all specimens showed macrocracks and microcracks.

159. A STUDY OF 1-DAY AND 3-DAY CALCULUS.—*Stanley P. Hazen, Eastman Dental Dispensary, Rochester, New York.* To study calculus formation, 30 mylar strips were placed on the lingual surfaces of the lower anterior teeth of 5 patients immediately after cleaning the teeth. The strips were removed at 1-day and 3-day intervals, fixed in 10 per cent neutral formalin, processed for celloidin imbedding, and sectioned and stained by various methods. Every specimen showed the presence of micro-organisms. At 1 day they were almost entirely cocci; by 3 days, they were still mostly cocci, but there were also many short rods and filamentous forms. The micro-organisms were scattered throughout the deposit and varied in location and quantity from specimen to specimen. Epithelial cells and leukocytes were also noted in every specimen. Most of these cells were seen on the surface, but some could be found in the depths of the deposit. No mineralization of the deposits was evident at the 1-day interval. By 3 days, however, strips from three of the patients gave evidence of mineralization. This degree of mineralization was reproduced on repeated strips. Two types of mineralization were evident: one, a surface precipitation form of mineralization and, two, a type that seemed to be a mineralization process in which there was evidence of a premineralized matrix around the developing area of mineralization. It also appeared that mineralization might have been occurring around bacteria in these areas. At 3 days, a definite pattern of calculus morphology could be noted.

160. AN ELECTRON-MICROSCOPIC STUDY OF CALCULOGENESIS.—*D. B. Scott and D. N. Wright, National Institute of Dental Research, National Institutes of Health, Bethesda, and H. A. Zander, Eastman Dental Dispensary, Rochester, New York.* Twenty-five 1-μ-thick mylar strips were fastened to the lingual surfaces of the lower

* Bio-Plastic, Ward's Natural Science Establishment, Inc.

anterior teeth of 4 individuals known to develop calculus rapidly. After 4 weeks, 14 strips were removed and placed in an osmium tetroxide–potassium dichromate mixture for fixation of the calculus-like material which had formed on them. Fifty pieces of the strip with deposit still in place were imbedded in methacrylate for sectioning with a microtime* equipped with glass and diamond knives. Sections were cut parallel to the mylar strip and observed in sequence under the electron microscope. It was found that, as first laid down, the deposit consisted primarily of micro-organisms of various morphologic types and an amorphous intermicrobial substance. The latter material seemed to be more dense immediately adjacent to areas of beginning mineralization. In regions of early mineralization, needle-like crystals, which gave electron diffraction patterns of apatite, were seen on the surfaces of and between the bacteria. Crystals were also found within the bacteria in regions of more advanced mineralization.

161. A Semiquantitative Measurement of Calculus Formation.—*Charles W. Smith and Gilbert J. Parfitt, School of Dentistry, University of Alabama, Birmingham.* A measurement of the amount of calculus formed and a means of distinction between calculus and food debris may be required in clinical investigations. A possible criterion is the degree of calcification of the deposits formed on the teeth. A method was devised whereby all the material removed from a particular tooth surface was examined microradiographically, the density of the films being measured on a densometer. Individual specimen carriers were made from Scotch tape and celluloid disks. A hole 3.5 mm. in diameter confined the specimen between the two layers of tape. The size of this aperture allowed exposure of the entire specimen. Exposure time and development were standardized so that the highest film density was within the range of the densometer scale. The aperture limiting the light source of the densometer was smaller than the field of the exposed film, so that extraneous light was excluded but still illuminated 80 per cent of the fielm. As the aggregates of calcified-material were not evenly dispersed, variations in the densometer readings were obtained by alteration of the position of the film. A minimum of three readings was therefore taken on each film. The amount of material that the X-rays could penetrate with standard exposure time was limited. This limit was reached in 2 out of 300 specimens collected from 150 individuals over a 4-week period. This procedure proved to be a simple semiquantitative method of determining the calcified material formed on the teeth.

162. Calcification Patterns and Inorganic Composition of Natural Dental Calculus.—*M. F. Little, J. Rowley, J. Barbera, and C. Cosciani, Eastman Dental Dispensary, Rochester, New York.* In an effort to establish criteria for the evaluation of artificial calculus, 782 pieces of natural dental calculus were examined for differences in calcification patterns revealed by soft X-ray and for corresponding differences in the organic and mineral content. Previously microradiographed samples of calculus weighing 0.1–1.5 mg. were individually placed in Cahn microplatinum boats, dried at 68° C. under vacuum, and ashed at 500° and 900° C. Aliquots of the dissolved residue were analyzed fo Ca and Na by flame photometry and for phosphorus by the formation of the molybdo-vanado-phosphate complex. The microradiographs showed a range of calcification within each piece which was not so great as that from piece to piece. Three major classifications were established based upon the relative radio-opacity of the calculus. The loss in weight on ashing (organic, CO_2, and water) ranged from 14 to 36 per cent and followed the previously assessed calcification pattern, with the highest losses from samples with the least radio-opacity. The phosphate and calcium values appeared to reflect similar calcification differences, whereas the sodium did not. The average Ca:P ratio was 1.75.

163. (Not presented.)

* Porter-Blum.

164. PYROPHOSPHATASE ENZYME AND ORAL CALCULUS.—*Aldona Prapuolenis and G. W. Rapp, Loyola University School of Dentisty, Chicago.* Pyrophosphatase is an enzyme which hydrolyzes pyrophosphoric acid and its soluble salts to orthophosphate. Its action makes it possible to increase the concentration of both calcium and phosphate ions at its site of action, thus overstepping the solubility product and permitting precipitation of calcium phosphate. Pyrophosphatase activity has been shown in salivary debris and in mixed cultures of oral micro-organisms. The debris spun down from 1 ml. of whole human saliva hydrolyzed 250 μg of pyrophosphate in 1 hour. Salivary micro-organisms grown in brain-heart infusion broth or thioglycolate medium split from 300 to 800 μg of pyrophosphate in 1 hour. Pyrophosphatase, acting on non-ionic, soluble calcium pyrophosphate, was shown to cause rapid precipitation of $CaHPO_4$.

165. RAT CONNECTIVE TISSUE REACTION TO IMPLANTED PERIODONTAL POSTOPERATIVE PACKS.—*Charles D. Hall and David F. Mitchell, Indiana University School of Dentistry, Indianapolis.* After surveying thirty-six dental schools, the nine most popular postgingivectomy dressings and a newly recommended one (bacitracin) were implanted in triplicate as freshly mixed pellets under the skin of 30 male Wistar rats. Gross observations were made, and one animal, with three implants, was sacrificed per material after 2, 16, and 32 days, and tissues around the implants were studied microscopically. The inflammatory reactions at each interval were classified as mild, moderate, or severe, according to number of leukocytes, degree of vascularity, and fibroplasia of the capsule, as in prior studies of pulp-capping agents and filling materials. The following packs (C = commercial product) gave uniformly mild reactions at all intervals: Ohio State, Orban, Ward's Wondrpak (C), Schlein's (C), and Rower (C). The packs recommended by Columbia, Tufts, and Indiana produced some mild and some moderate reactions. Professional Products (C) and the bacitracin pack were uniformly moderate in response. No severe reactions occurred. It is believed that this test can serve in the future for screening newly proposed periodontal packs for their irritational qualities.

166. TISSUE REACTIONS TO IMPLANTED POSTOPERATIVE DRESSINGS.—*Richard G. Scering and David F. Mitchell, Indiana University School of Dentistry, Indianapolis.* Thirty-eight dental schools named twenty-nine different drugs used for treatment of "dry socket." Twelve were selected as most repesentative, and each was implanted in triplicate, subcutaneously, in 6 male albino rats. Through skin incisions, approximately 0.5 ml. of material was placed, and the wounds were sutured. After 2, 16, and 32 days, the animals were sacrificed, providing six specimens per material for each period. The gross and microscopic tissue reaction around the drugs was graded as mild, moderate, or severe, based on thickness and vascularity of the capsule, number of leukocytes, and presence of abscesses and fistulae. The following showed uniformly mild reactions at each period: Ward's Wondrpak Surgical Cement, iodoform gauze, eugenol on iodoform gauze, saline on gauze, and Camphophenique on gauze. Bipp's and Pell's pastes on gauze and Dentalone on gauze each showed a moderate reaction at one interval and were mild at the other two. Guaiacol on iodoform gauze was moderate, moderate, mild; Butyn Metaphen Dental Ointment was severe, moderate, moderate; and Getz-400 Surgical Dressing was severe, severe, severe.

167. SUBCUTANEOUS CONNECTIVE TISSUE REACTION OF RATS TO IMPLANTED DENTAL CEMENTS.—*John B. Boyd, Jr., and David F. Mitchell, Indiana University School of Dentistry, Indianapolis.* In continuing efforts to assay the relative irritant qualities of various dental materials and of various brands of these materials available commercially, nineteen phosphate and resin cements were implanted subdermally in 57 Wistar rats. Freshly mixed cements were injected subcutaneously. Gross reactions were observed, and tissues for histological study were obtained 2, 16, and 32 days after implantation. All responses were inflammatory and were classified as mild(M),

moderate(Mo), or severe(S) at each interval, depending on thickness of capsule, number of leukocytes, and degree of vascularity. Zinc phosphate cements generally produced mild to moderate reactions. Cements containing more zinc oxide and less additives produced the milder responses. It appeared that when silver, copper salts, silicates, or antibiotics were added, more severe responses were elicited. The findings correlated to a reasonable degree with the reactions of the dental pulp found by other investigators. Reactions in increasing order of severity(approximate) were: Stratford-Cookson, M,M,M; Caulk's Grip, Mo,M,M; S.S. White Zinc, Mo,M,Mo; Caulk's Tenacin, Mo,M,Mo; Justi Resin, S,M,M; Amco Lifetime, Mo,Mo,Mo; Caulk's White Copper, Mo,Mo,Mo; S.S. White Silver, S,Mo,Mo; and Lang's C. & B., S,Mo,S. The following products were uniformly severe at all intervals: Caulk's Black Copper, Fleck's Red Copper, Ames Black Copper, A.B.C. Polyantibiotic, Caulk's Di-Cem #1, S.S. White Kryptex, and Petralit. Obviously, this grouping is somewhat arbitrary, to the degree that individual cements cannot be sharply separated from their immediate neighbors.

168. (Not presented.)

XV. ENAMEL AND DENTIN—PHYSICAL AND CHEMICAL PROPERTIES

MARCH 19, AFTERNOON, SECTION B

169. THE NATURE OF THE PROTEIN MATRIX OF HUMAN ENAMEL.—*K. A. Piez, National Institute of Dental Research, National Institutes of Health, Bethesda.* Although published analyses of enamel protein show wide variability, they agree in that all the samples are reported to contain large amounts of glycine, proline, and hydroxyproline. From these data it might be concluded that enamel protein belongs to the collagen class. However, the possibility remains that all the samples may have been contaminated with large amounts of dentin collagen. To obtain further evidence on this point, enamel protein was carefully isolated from unerupted third molars and complete amino acid analyses were performed. The protein was nearly all soluble in the decalcifying solution (ethylenediamine tetra-acetic acid). The soluble protein contained no hydroxyproline or hydroxylysine and only moderate amounts of glycine, indicating that it was not a collagen. Its composition showed several distinctive characteristics but was unlike any other known protein. The small insoluble residue was similar in composition to the soluble protein if the presence of about one-third collagen was assumed. Thus it seems probable that previous difficulties with the analysis of enamel protein were the result of contamination with dentin collagen, as well as losses of enamel protein because of its solubility.

170. STUDIES ON FORMATION AND MINERALIZATION OF ENAMEL MATRIX.—*J. K. Avery and R. L. Visser, University of Michigan School of Dentistry, Ann Arbor.* Studies on the development and mineralization of enamel matrix have been carried out on human and rhesus monkey teeth, using microradiography, microhardness measurements, and electron microscopy. The early phases of enamel mineralization were studied by microradiography, whereas the later phases of harder enamel were studied by microhardness tests. Although the interprismatic substance of the enamel was formed and organized before the substance of the enamel rod was formed, the enamel rod was calcified before the interprismatic substance. The organic fiber components of the enamel rods developed in the long axis of the rods and approximately at a 45° angle to the direction of the ameloblasts. The first mineralization seen in the young enamel matrix appeared as a narrow but distinct band at the dentinoenamel junction. A small amount of mineralization then appeared to spread diffusely throughout the enamel matrix of the cusps. No incremental lines appeared in the microradiographs in the earlier stages of enamel mineralization. As the mineral content of the cusps increased slightly, incremental lines became apparent as hypermineralized lines in comparison with the surrounding matrix. As the incremental lines appeared, continued mineralization seemed to be incremental; thus the increments were progressively less mineralized from the dentinoenamel junction peripherally to the surface

of the enamel. One of the later stages of enamel mineralization was the calcification of the interprismatic substance. Before this stage of mineralization the calcified enamel rods could be dissected apart by removing the highly organic interprismatic substance with ethylenediamine. During the last phases of enamel mineralization, rods could not be separated by this method, and it was concluded that the interprismatic substance was calcified. It is possible that, during the mineralization of the enamel-rod matrix, inorganic ions circulated throughout the interprismatic substance.

171. A Study of Zn in Human Teeth.—*F. Brudevold and L. T. Steadman, Forsyth Dental Infirmary, Harvard School of Dental Medicine, Boston, and University of Rochester, Rochester, New York.* The distribution pattern of Zn in teeth was found to be similar to that of F, and molar concentrations of the two elements were frequently of the same order. In enamel, the major deposition of Zn took place before tooth eruption. In contrast to F, posteruptive deposition of Zn appeared to be erratic. Concentrations of Zn in surface enamel of teeth from different areas ranged from 430 to 2,100 ppm. Zn was readily acquired by powdered enamel and by synthetic hydroxyapatite. Ca competed with Zn for positions on the surface of the apatite crystals.

172. An Anomalous Condition of Human Cervical Enamel.—*Barbara J. Wallace and Thomas B. Coolidge, University of Chicago.* The enamel of the cervical region of many teeth appeared more opaque than that in other regions of the tooth when undecalcified sections were viewed in reflected light. Observation with the polarizing microscope showed these cervical regions to be circumscribed spindle-shaped areas exhibiting strong positive form birefringence. The condition was generally more closely associated with the dentinoenamel junction than with the outer enamel surface. During imbibition the behavior of such regions was like that of the anomalously reacting "positive" zone of the carious lesion. These areas appeared to be partially demineralized or improperly mineralized regions. Their frequent occurrence in unerupted third molars suggested that they were not (incipient) caries. The anatomical changes noted in this region of the enamel were described as seen in the polarizing and phase-contrast microscopes. Some similarities and dissimilarities to carious lesions were discussed.

173. The Crystal Chemistry of Dental Enamel.—*Duncan McConnell, Ohio State University, Columbus.* Since my proposal (1952) of a modified crystal structure for the substance of dental enamel and bone, significant experimental data have appeared as a result of the efforts of several persons. Some of these data offer very strong confirmatory evidence of the correctness of the basic postulate: carbon replaces phosphorus in the crystal lattice of apatite. Moreover, these data indicate that, despite the small size of the crystallites which comprise dentin and bone, the carbonate is not adsorbed to any appreciable extent on the surfaces of the crystallites but can be entirely contained within the apatite lattice. Dental enamel produces an excellent powder-diffraction pattern and serves as a most important analogue when comparing the minerals dahllite and francolite. The unit cell dimensions of these substances do not vary directly with the fluorine or carbon dioxide contents but seem to depend principally upon the quantity of structurally combined water other than the hydroxyls replacing fluorine.

174. Microscopic Studies of Abrasion and Attrition on Tooth Surfaces.—*Albert A. Dahlberg, Zoller Memorial Dental Clinic, University of Chicago.* Abrasion and attrition induced characteristic markings on tooth surfaces that were distinctive at the microscopic level. Marks from abrading materials on occlusal surfaces were usually directionally oriented with mandibular movements and resulted in myriads of miniature gothic arch tracings. Scratches and markings on other surfaces did not show this sort of organization except in specific instances. Attrition without abrasive materials left a polished unmarked surface which was readily distinguished from the abraded ones. Scratches and markings reflected the nature of the abrading agent. They were long, short, deep, shallow, straight, curved, interrupted, light, heavy, deviant,

dividing, single, double, parallel, intersecting, gouged, clean, sharp, vague, or split. They were old and faint in the background or overlay subsequent, more recent, and sharper markings, representing several levels. Sand, for example, produced scratches of irregular character which differed from the regular clean cuts of smaller and sharper particles, such as carborundum. Replications of tooth surfaces of entire dentitions could be obtained by the application of a clear, fine detail, reproducing a non-solvent type of replicating material such as polyvinyl alcohol in rubber base impressions. More detail at levels below 10 μ was obtainable by direct collodion replicating technic.

175. Isolation and Partial Fractionation of Fluorescent Material from Human Teeth.—*Ulla-Riitta Laurila and Sandra A. Mancewicz, Naval Medical Research Institute, Bethesda, and A. F. Forziati, Research Division, National Bureau of Standards, Washington, D.C.* Fluorescent protein complexes were isolated from dentin and from roots of human teeth by the following methods: (1) dentin was demineralized with 0.1 N HCl, at 4° C., for 3–4 days, or with 5 per cent EDTA, pH 7.5, at 25° C. for 3 days; (2) dentin was extracted with boiling water for 24 hours, and the water-soluble protein precipitated with 75 per cent ethyl alcohol; (3) roots were extracted with ethylene glycol–3 per cent KOH for 2–3 hours, and the resulting solution extracted with ether, chromatographed on charcoal/cellulose (1:1) and on Dowex-1-X2. These methods yielded substances which fluoresced blue under ultraviolet light ($\sim$365 mμ). The maxima of the activation and fluorescence spectra were observed at 328–30 and 395–400 mμ, respectively, measured with a spectrophotofluorometer.* Each substance was hydrolyzed with 6 N HCl (105° C., 20 hours) in sealed tubes. Paper chromatography of each of the hydrolysates, Whatman No. 1 and No. 3, *n*-butanol/formic acid/water (75:15:10), revealed a fluorescent spot at the application point. Ninhydrin and specific reactions applied to paper chromatograms, developed in various solvents, indicated the presence of amino acids from the dentinal protein. Approximate quantitative estimations of amino acids were made according to the method of Polson *et al.* Ca and P contents were determined. Chymotrypsin, trypsin, papain, pepsin, and collagenase did not release the fluorescent pigment in appreciable amounts, but they liberated some peptides from the protein complexes. Partial hydrolysis of the complex from method 3 (11.2 N HCl, 37° C., 7 days) yielded a number of short peptides, the least mobile of which contained the fluorescent pigment. 6 N HCl hydrolysates of preparations 1 and 3 were evaporated under reduced pressure; the residues were chromatographed on charcoal and the pigment eluted with hot pyridine. Subsequent paper chromatography, in acidic and basic solvents, revealed only one major fluorescent spot from each preparation.

176. On the Three-dimensional Morphology of Crystallites in Human Bone and Dentin.—*Erling Johansen and Harold F. Parks, Departments of Dental Research and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, New York.* In sections of human dentin (carious and sound) and alveolar bone examined with the electron microscope, apatite crystallites were seen to present long, thin profiles somewhat suggestive of a cylindrial shape, broad profiles indicative of a platelike shape, and profiles intermediate between these two extremes. With a stereoscopic technic it was possible to record images of two profiles of the same crystallite from different angles. All thin-profile crystallites so examined, that were properly oriented with respect to the axis of tilt, exhibited a profile of different width in each of the two views and were thus demonstrated to be platelike rather than cylindrical. The approximate maximum (length) and minimum (thickness) dimensions of these crystallites were as follows: 40 A thick and 700 A long in carious dentin; 35 A thick and 1,000 A long in normal dentin; and 40 A thick and 1,000 A long in bone. These observations were discussed in relation to conflicting data regarding the size and shape of crystallite units obtained by different methods.

* Aminco-Bowman.

177. MICROCHANGES IN SOUND HUMAN DENTIN.—*I. A. Mjør, M. B. Quigley, and S. B. Finn, University of Alabama, Medical Center, Birmingham.* A study has been conducted to determine the effect of $Ca(OH)_2$ and amalgam on non-carious young dentin. Twenty cavities were prepared in non-carious permanent teeth, which were to be extracted later for orthodontic reasons. Some were filled with a base of $Ca(OH)_2$ while others were filled with amalgam only. When possible, a non-operated tooth from the same patient was used as another control. After extraction the teeth were imbedded in plastic and sectioned longitudinally at the mid-point of the filling. Longitudinal sections were cut from one half, and those nearest the center of the filling were used for microscopic, microradiographic, and subsequent densitometric studies. On the other half-block, microhardness tests were carried out, after which cross-sections were cut and the same studies carried out on those as on the longitudinal sections. The $Ca(OH)_2$-covered dentin showed a significant increase in microhardness as compared with (1) unaffected dentin in the same tooth, (2) dentin in unoperated teeth, and (3) dentin areas under amalgam fillings. The increase in hardness under amalgam fillings as compared with unaffected dentin in the same tooth was variable and does not appear to be significant. Microradiographic and densitometric studies substantiate the results obtained by microhardness studies.

178. X-RAY AND ELECTRON MICROSCOPY OF SECONDARY CALCIFICATION WITHIN AGING DENTAL TUBULES.—*John Nalbandian, Harvard School of Dental Medicine, Frederico Gonzales, University of Texas Dental Branch, Houston, and Reidar F. Sognnaes, Harvard School of Dental Medicine.* During primary development of mesenchymal hard tissues, the collagenous matrix is subject to rapid phosphate deposition in close juxtaposition to the formative surfaces of osteoid and predentin (Sognnaes *et al., Am. J. Phys.*, **180**:408, 1955). On the other hand, the secondary mineralization in sclerotic root dentin of human teeth (Nalbandian, *J. D. Res.*, **38**:681, 1959)—which we examined by microradiography of thin ground sections and electron microscopy of undecalcified specimens cut with a diamond knife—is characterized by a prolonged period of crystal deposition at a considerable distance from the mesenchymal pulpal cells. Ultimately, this intratubular process led to a very high microradiographic density (much greater than that of intertubular dentin), completely obliterating the dentinal tubules and producing an optically transparent tissue, the resulting mineral distribution being uniform enough to avoid scatter of transmitted light. The inorganic crystals exhibited a selective area electron defraction pattern of apatite both within and between the sclerotic dentinal tubules, notwithstanding the differences in origin, quantity, and quality of the organic templaes. The implication of this and other types of secondary apatite nucleation in different organic matrixes within and around the teeth were discussed in relation to current theories on calcification.

179. DISTRIBUTION OF NATURALLY OCCURRING RADIOACTIVE ISOTOPES IN HUMAN TEETH AS SEEN IN AUTORADIOGRAMS.—*A. M. Hunt, University of Toronto.* Carbon^{14} and probably potassium^{40}, tritium, and other naturally occurring radioactive isotopes are present in human teeth. An attempt was made to determine their presence and distribution by means of autoradiograms. Pre-1945 teeth were used to eliminate the possibility of radiation from bomb fission products. 300-μ ground sections were dipped in liquid emulsion* and exposed for 5 months. Most of the detectable radiation in Dentin was present in the dentinal tubules. Few silver grains appeared between tubules. In enamel, radiation came only from the enamel lamellae. Partly decalcified tooth slices revealed a more diffuse and intense pattern of grains from the same sites. The presence of tracks from beta particles suggested that C^{14} and perhaps K^{40} were present. The grain pattern did not illustrate the known distribution of carbon. It is possible that the higher self-absorption of C^{14} in the denser calcified material in between dentinal tubules and in the enamel may account for the observed distribution.

* Kodak N.T.B.2.

180. Electron-microscopic Study of Lathyritic Rat Incisors.—*R. R. Bruns, University of Rochester School of Medicine and Dentistry, Rochester, New York.* Osteolathyrism is known to cause profound alterations in connective tissue, but the exact nature of this change has not yet been determined. One of the outstanding features of a lathyritic animal is the weakness or fragility of its connective tissue. This fragility may be due to a defect in the formation of the collagen fibers or to breakdown of collagen fibers already formed, as suggested by Levine and Gross (*J. Exptl. Med.*, **110**:771, 1959). In the present study predentin collagen was examined with the electron microscope to determine whether lathyrism caused the formation of abnormal-appearing collagen fibers. The predentin was selected because collagen, which was known to have formed while the rats were under the influence of the lathyrogenic agent, could be examined. Weanling rats were made lathyritic by the subcutaneous injection of 40 mg. B-aminopropionitrile fumarate twice a day for 3 days. The apexes of mandibular incisors were removed, fixed in buffered 1 per cent OsO_4 at pH 7.4, sectioned, and examined with an electron microscope.* The collagen fibers present in the predentin of both lathyritic and control rats showed the typical banding characteristic of normal collagen. From the material examined to date it seems that normal-appearing predentin collagen can be formed by lathyritic rats.

181. Dentin Apposition in Albino Rat Molars.—*Leif B. Johannessen, Forsyth Dental Infirmary, Boston.* Dentin apposition in mandibular first molars was studied in 96 female and 96 male rats. A 0.2 per cent solution of sodium fluoride was injected intraperitoneally at 31, 41, and 61 days of age. The first two injections were 20 mg F/kg of body weight, the third was 10 mg F/kg of body weight. Forty of the females and males served as controls. The animals were sacrified when 91 days old. Steel matrixes were used for aligning and imbedding 8 to 10 molars in a row according to their buccolingual diameter. An epoxy resin† was used as imbedding material. A predetermined equal amount of material was removed from the buccal and lingual sides by means of a micrometer stage grinder. The finished sections were 95–105 μ thick. On standardized tracings of the teeth, the mesial and distal dentin widths were measured along a straight line connecting the mesial and distal cementoenamel junctions. The occlusal dentin apposition was measured on a perpendicular to this base line from the most apical point of the dentinoenamel border in the centro-occlusal groove. The preliminary results showed that there was a rapid rate of dentin apposition during crown and root formation, as well as for the following 40–70 days for the proximal and occlusal dentin, respectively. Thereafter, the dentin apposition was greatly decelerated.

XVI. SALIVARY STUDIES. MARCH 19, AFTERNOON, SECTION C

182. Endogenous Oxygen Uptake Activity of Human Saliva.—*A. Szabo, P. Howard, R. Rafferty, and B. Eichel, Institute of Stomatological Research, Sias Research Laboratories, Brooks Hospital, Brookline, Massachusetts.* Whole, paraffin-stimulated saliva samples were collected directly into cold buffer solution. The rapid oxygen utilization of the buffered saliva in the absence of added substrate indicated that the saliva contained large amounts of aerobically metabolizable endogenous substrates. The variability of whole-saliva oxygen utilization for identical flask systems was as great as $\pm$ 75 per cent when 0.1 M PO_4, pH 7.4, was employed as the buffer medium. The variability of identical flask systems was reduced to levels as low as $\pm$ 7 per cent, however, in the presence of 0.1 M glycyl-glycine buffer, p H7.0. The effect of 0.001 M cyanide on endogenous whole-saliva oxygen uptake in the presence of glycyl-glycine buffer was studied. Inhibitions of 75 and 50 per cent, respectively, were obtained when 20 and 100 per cent oxygen atmospheres were used, suggesting the participation of flavoprotein oxidase and cytochrome oxidase–like mechanisms.

* RCA EMU 2A.

† Hysol 6020.

The addition of glucose to these whole-saliva mixtures sometimes slightly activated, or inhibited, or produced no effect upon the oxygen consumption. Oxygen-uptake assay of high-speed centrifuged sediment and supernatant fractions prepared from whole saliva clearly demonstrated that the endogenous whole-saliva respiration was dependent on the presence of sediment and suitable amounts of the supernatant fractions. The sediment fraction is heat-labile, while the supernatant fraction is heat-stable. Fortification of the sediment with glucose, DPN, TPN, cytochrome *c*, and ATP in the presence of Krebs-Ringer buffer did not elicit oxygen consumption by the sediment.

183. Parotid Secretion Rate for Measuring Response to Gustatory and Masticatory Stimuli.—*H. H. Chauncey, Tufts University School of Dental Medicine, Boston, and I. L. Shannon, School of Aviation Medicine, USAF.* The procedures described present a new approach to the quantitative determination of the effect of gustatory and masticatory stimuli. The action of masticatory stimuli was evaluated by means of gum base, paraffin, and rubber bands. The effect of the four gustatory modalities (sour, salt, sweet, and bitter) was determined by using solutions of citric acid, sodium chloride, sucrose, and quinine. Solutions were applied with wetted cotton applicator sticks. Parotid saliva was obtained with a vacuum cup and collected in graduated tubes. Glandular secretion rate was observed to be a linear function of the logarithm of bolus volume for masticatory stimuli and application rate (swabs/min) for gustatory stimuli. Parotid flow rate was only slightly affected by chewing rate. Increasing concentrations of gustatory stimuli resulted in a secretory response which was linear throughout the entire series for sucrose and quinine but was linear only at lower concentrations with citric acid and sodium chloride. Mathematical expression of the relationship between glandular secretion rate, bolus volume, and application rate permitted calculation of maximal response and reflex equilibrium constants for the various stimuli. The results indicated that at least three gustato-salivary reflex pathways are involved.

184. Pyruvic Reductase Activity—Whole Human Saliva and Saliva Supernatant Preparations.—*B. Eichel, K. Skolnick, and V. F. Lisanti, Institute of Stomatological Research, Sias Research Laboratories, Brooks Hospital, Brookline, Massachusetts.* Pyruvic reductase (lactic dehydrogenase) activity has been demonstrated in human saliva of children and young adults by spectrophotometrically following the oxidation of reduced diphosphopyridine nucleotide (DPNH). The enzyme activity has been quantitated in whole saliva and in supernatant fractions prepared from whole saliva. Exogenous enzyme activity was invariably greater than endogenous activity. Supernatant fractions prepared from stimulated salivas, collected during chewing of paraffin or rubber bands, consistently yielded higher total, endogenous, and exogenous activities, than did supernatant fractions prepared from unstimulated salivas. Since parotid and submaxillary salivas were free of enzyme activity, the likely source of the enzyme in the supernatant fractions was the saliva sediment. The higher enzyme activity of the stimulated salivas was probably due to the removal of deposits from teeth, exudates from gingival crevices, and epithelial cells from the mucosal tissues by the mechanical action of the chewing agents employed. The pyruvic reductase in the supernatant saliva fractions was partially characterized. Increasing the sample size of the saliva collection by volume did not result in a dilution of the activity of supernatant fractions. The inhibition of the enzyme activity by suitable concentrations of cyanide in the presence of added substrate supported the identity of the enzyme. The enzyme activity was stable over a 3-day period and lost only 33 per cent of its activity when dialyzed against cold distilled water for 24 hours. Fifty to 85 per cent of the salivas' capacity to convert pyruvic acid into lactic acid was localized in the soluble phase of the whole saliva. This finding strongly suggested the need to modify the current theory that lactic acid production is associated only with the sediment fraction of saliva and its contained bacteria.

185. The Protein-bound Carbohydrates of Parotid Saliva.—*I. D. Mandel and B. Hampar, School of Dental and Oral Surgery, Columbia University, and S. A. Ellison, College of Physicians and Surgeons, Columbia University, New York.* Analyses were made of protein-bound carbohydrates in stimulated human parotid saliva. Proteins precipitated with phosphotungstic acid (5 per cent in 2 N HCl) were assayed for hexose (anthrone reaction), hexosamine (Elson-Morgan), and fucose (Dische-Shettles cysteine reaction). The total amounts of these (mg. per cent) were as follows: hexose, 11–54 (av. 26); hexosamine, 4–18 (av. 7.5); and fucose, 2–10 (av. 5). Small amounts of sialic acid and deoxyribose were also found. The carbohydrates were then separated and identified, and the proteins to which they were bound were characterized. Lyphilized saliva samples were redissolved in 1/50 their original volume of 1 M NaCl, dialized against water, and then hydrolyzed in 0.1 N HCl for 1 hour. The hydrolysates were dialyzed again, and the dialyzable materials were collected and concentrated. For hexosamine determination, the concentrated dialyzate was chromatographed on Dowex-50, eluting with 0.3 N HCl. Assay of the eluates for reducing substances indicated the presence of two separate components. These fractions, after actylation and reaction with Ehrlich's reagent, were identified as *N*-acetylglucosamine (85 per cent) and *N*-acetylgalactosamine (15 per cent). Neutral sugars were separated on a mixed resin and identified by paper chromatography (propanol-ethanol-water, 7:1:2; triple ascension). They consisted of galactose, mannose, and fucose. Analysis of salivary proteins isolated by elution from paper strips after electrophoresis indicated that most of the hexose and hexosamine and essentially all of the fucose were associated with two of the basic proteins.

186. A Glucose-Tolerance Test Employing Parotid Secretion.—*R. B. Wolcott and T. B. Weber, Dental Research Facility, Great Lakes, Illinois.* In an attempt better to understand the carbohydrate metabolism of the parotid gland concomitant with the increased importance of oral manifestations of diabetes mellitus, a glucose-tolerance curve was established on the pure secretion from that gland. Ten healthy, postabsorptive individuals whose baseline was well established were given single doses of glucose to the extent of 1 gm/kg body weight. Measurements were made at varying intervals up to 3 hours after ingestion. Stimulation was effected by chewing flavored chickle gum, and collections were made with conventional parotid cups. Care was taken to prevent any chemical or bacteriologic contamination. A modified anthrone technic of sufficient sensitivity to detect minor changes was employed. The period of rise and persistence of glucose was similar to that found in blood, although the initial rise was more rapid and the duration slightly longer. The amount of glucose present was generally much less than that found in blood, although its initial appearance was immediate after dosage. It was necessary to make collections for 15 minutes in order to obtain replicate values. Other sugars and commonly employed commercial sugar substitutes were also tested, with favorable results. This technic eliminates the distressing, cumbersome sampling means now employed to measure glucose tolerance by voluminous urine samples or repeated venipunctures. It also affords the dentist an opportunity to work solely in the oral cavity, a region with which he is familiar and for which he is equipped.

187. Carbohydrate in Parotid Secretion.—*T. B. Weber, V. J. Lux, and I. L. Shklair, Dental Research Facility, Great Lakes, Illinois.* A highly sensitive technic employing anthrone was devised to measure the difference in the amount of carbohydrate secreted from caries-immune and caries-rampant individuals. This method measured probably all carbohydrates except the uronic acids and amino sugars. Each extreme group contained the secretions of 20 young adult males in the naval service who lived in a similar environment and subsisted on the same diet. The immune group was shown to be free of caries by clinical and radiologic examination, while the rampants had DMF values of 15 or greater. Collections were made in a postabsorptive state with conventional parotid cups and were free of any bacteriologic or chem-

ical contamination. Stimulation was by chewing flavored chickle gum under a controlled regimen. The mean and standard error for the immunes and rampants was 18.1 ± 0.5 and 60.2 ± 12.4 mg. per cent, respectively. These values suggested that, even though the amount of carbohydrate secreted by the glands was minute, there was, nevertheless, more than three times as much in the carious group. This becomes significant when the total amount continually bathing the surfaces of the teeth is considered, as it may serve as an energy source for the oral microbiota. A glucose oxidase test, specific for glucose, was also run on each group, and the glucose oxidase/anthrone ratios compared favorably.

188. Relationship of Carbohydrates and Tryptophane to Salivary Viscosity, Flow, and Caries in the Rat.—*Richard P. Elzay and Joseph C. Muhler, Department of Biochemistry, Indiana University Medical Center, Indianapolis.* Studies have reported evidence that the amino acid tryptophane may be associated with dental caries. Since carbohydrate degradation is believed to be involved in tryptophane metabolism and since increased levels of carbohydrate in the diet affect salivary viscosity, it was decided that investigations concerning the effect of carbohydrates and tryptophane on the teeth and salivary glands of laboratory animals would be of interest. Salivary flow and viscosity were used as a measure of salivary function. Salivary samples were analyzed for total protein and tryptophane content, and the heads were evaluated for dental caries. The data indicated that increased levels of carbohydrates in the diet were associated with a decreased weight gain and an increase in salivary viscosity. There was no significant difference in flow. The protein content of saliva increased with the addition of tryptophane to the diet. The caries activity was decreased by the addition of tryptophane to the diet.

189. Salivary Excretion of Sulfisoxazole.—*Bernard M. Carr and Donald C. Kroeger, University of Texas Dental Branch, Houston.* Previous work has shown that the sulfonamides are excreted in the mixed salivas of both dog and human. Little work, however, has been done to indicate the extent and the time-course relationship of the appearance of sulfonamides in the excretions from the individual glands. This study consisted of determining the concentration of sulfisoxazole diethanolamine (Gantrisin®), injected intravenously, appearing in the parotid, the submaxillary, and mixed salivary samples from ten anesthetized dogs stimulated with pilocarpine. Salivary volumes, as well as blood and urine concentrations and volumes, were determined every half-hour for 4 hours. The observed disappearances from blood and the appearance in the urine compared favorably with the published reports for other sulfonamides. After an initial drop during the first 30 minutes, total amounts of sulfonamides appearing in the three salivary samples remained constant for the next 3½ hours, even though concentration and volumes varied widely. Contrary to a current hypothesis stating that the sulfonamides are excreted by simple diffusion, the evidence obtained suggested a secretory mechanism largely independent of blood concentrations and salivary flow rate.

190. Phagocytosis in Vitro by Salivary Corpuscles.—*G. H. Rovelstad and J. C. Calandra, Northwestern University Dental School, Chicago.* Fresh saliva mixed with an infusion broth containing glucose at pH 7.8 and sealed in a chamber in an incubator at 37° C. was observed and photographed through a bright-phase-contrast microscope. Salivary corpuscles were found to become markedly active with typical amoeboid movement. A homogenous substance was noted to flow in advance of a pseudopod. The remainder of the cell then streamed into this field and thus affected movement. The act of phagocytosis included the above movement, with the cell overflowing the bacteria and incorporating them intracellularly. The above phenomena were observed and recorded by means of time-lapse phase-contrast photomicrograph and were suggested as in vitro evidence of a functioning scavenger mechanism in the oral cavity.

191. Histochemical Studies on Enzymes of Salivary Corpuscles.—*H. O. Yokoyama, G. H. Rovelstad, and J. C. Calandra, Northwestern University Medical and Dental Schools, Chicago.* Salivary samples and saliva smears were collected from ten young male adults at 10 o'clock in the morning after 2 hours of fasting. All subjects were free from any evidence of dental disease of the hard or soft tissues of the oral cavity at the time of the sampling. Histochemical methods were employed on smears to observe the presence of several enzymes in the salivary corpuscles normally found in saliva sediment. High activity was noted for peroxidase, acid phosphatase, alkaline phosphatase, and peptidase in most cells. Positive reactions for nonspecific esterase and succinic dehydrogenase were observed in some cells. These observations on salivary corpuscles were compared with those of leukocytes from peripheral blood.

192. Electron-microscopic Study of Parotid Gland Secretion in Mice and Rats.—*Harold F. Parks and Erling Johansen, Departments of Dental Research and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, New York.* White mice were given pilocarpine hydrochloride (10 mg/kg body weight) intraperitoneally or subcutaneously. Also, mice were given food after a 24-hour period of starvation. Parotid glandular tissue was taken at various intervals following each type of secretion stimulus and prepared for electron-microscopic examination. Three types of secretory activity were seen in acinous cells: (1) extrusion of contents of secretion granules, (2) extrusion of contents of cytoplasmic vacuoles, and (3) pinching-off of cytoplasmic blebs. In the first two types the membrane inclosing the granule or vacuole became continuous with the plasma membrane around a constricted opening that allowed the secretory contents to flow into the lumen of acinus or intercellular secretory canaliculus. While secretory granules are normally present in the resting cell, vacuoles were formed only in response to a secretory stimulus, mainly by swelling and/or coalescence of secretion granules. The cytoplasm immediately surrounding some vacuoles was of very low density, suggesting an increased water content. Segments of endoplasmic reticulum extending into this zone of altered cytoplasm were sometimes swollen and degranulated in pilocarpine-stimulated cells. Striated duct cells formed large apical cytoplasmic blebs of very low density. In the mouse, hunger alone sufficed to cause secretion of granules in acini and extensive bleb formation in striated ducts; feeding after starvation caused vacuole and bleb formation in acini. Pilocarpine effects in mouse and rat were qualitatively similar to, but stronger than, those resulting from natural stimuli.

193. Serum and Parotid Fluid Interrelationships.—*Ira L. Shannon and John R. Prigmore, School of Aviation Medicine, USAF, Brooks Air Force Base, Texas.* The concentrations of glucose, sodium, potassium, and chloride were determined in serum and parotid fluid collected from 15 healthy young adult males under glucose-tolerance (100 gm. orally) conditions. Glucose was determined on the Autoanalyzer, and a measurable quantity was found in all parotid fluid samples. The method was accurate to concentrations of less than 0.5 mg. per cent glucose. The results are shown in the table. Parotid fluid glucose levels were seen to change under tolerance condi-

Time after Dosage (Hours)	Flow Rate*	Parotid Fluid				Serum			
		Na	K	Cl	Glucose	Na	K	Cl	Glucose
0	0.815	38.4	20.1	29.0	1.2	146	4.9	100	88
½	0.702	38.5	20.3	25.4	1.5	146	4.8	101	149
1	0.767	40.3	20.8	26.3	1.5	146	4.8	101	125
2	0.833	39.3	31.0	27.2	1.4	146	4.8	101	103
3	0.842	40.5	20.9	30.1	1.3	146	4.9	100	81

* Flow rate is in ml/min; Na, K, Cl in mEq/l; glucose in mg. per cent.

tions, but very little glucose passed from the blood to the parotid fluid at these serum concentrations. Twenty-two subjects were given oral doses of 60 gm. of urea; 12 were followed for 1 hour and 10 were sampled for 3 hours. Parotid fluid and serum urea were determined automatically by a modification of the carbamidodiacetyl reaction. Urea diffused very quickly from the blood stream to the parotid fluid, and, at all levels of concentration, the parotid urea reflected the level found in the serum. Parotid fluid is thus amenable to medical laboratory procedures for uremia diagnosis. Contrary to existing reports, a flow-rate and urea relationship were found for parotid fluid.

XVII. CARIES—ANIMAL STUDIES. MARCH 20, FORENOON, SECTION A

194. THE EFFECT OF ADRENALECTOMY ON EXPERIMENTAL DENTAL CARIES.—*Edward A. Sweeney, Harvard School of Dental Medicine.* This experiment was designed to determine the influence of adrenalectomy with or without cortisone replacement on dental caries incidence in this laboratory's strain of caries-susceptible rats. One hundred and twelve animals were distributed at weaning among the following five groups by litter and sex: (I) control rats maintained on water and cariogenic diet 700; (II) control rats provided 0.9 per cent NaCl solution and ration 700 + 0.6 per cent NaCl; (III) adrenalectomized rats fed the same as rats in Group II; (IV) rats maintained on ration 700 and water, but given 0.6 mg. of cortisone acetate daily by subcutaneous injection; and (V) adrenalectomized rats maintained as in Group IV. Adrenalectomies were performed at 28 days, and the animals were sacrificed at 100 days. The animals in groups provided with additional salt drank approximately twice as much as did those in Group I. Rats given cortisone drank approximately the same as the Group I animals. Food consumption was approximately the same in all groups. No statistically significant difference in dental caries was noted among the five groups. Body weights of males in Groups I and II at sacrifice were comparable, as were the weights of the females in Groups I, II, III, and IV. Males in Group IV weighed about 10 per cent less than those in Groups I and II. Males in adrenalectomized Groups III and V weighed 20 per cent less than those in Group I, as did the females in Group V as compared with Group I.

195. STUDIES CONCERNING MECHANISM OF GOITROGENS AND DENTAL CARIES.—*William G. Shafer and Joseph C. Muhler, Indiana University, Departments of Oral Pathology and Biochemistry, Medical Center, Indianapolis.* Studies from our laboratories have consistently shown a relationship between experimental dental caries, thyroid activity, and the major salivary glands in rats. Hypothyroidism has invariably resulted in a significant increase in caries incidence and alterations in salivary gland structure. Hyperthyroidism reduces caries as well as increases the size and number of salivary gland granular tubules. To learn more about this mechanism and to determine whether other antithyroid drugs act similarly, thiocyanate was administered at different levels to groups of rats, and the effect on salivary flow and viscosity, dental caries, and salivary glands was studied. For comparison, two other recognized goitrogens—methimazole (1-methyl-2-mercaptoimidazole) and dinitrophenol—were also used, as well as propylthiouracil and a proteinase. The results showed that when potassium thiocyanate was stomach-tubed at a level of 5 mg. per rat per day there was an increase (10.2 lesions) in dental caries, but when stomach-tubed at a level of 50 mg. a day there was no change (8.2) in the caries incidence when compared with controls (8.2). Dinitrophenol had no effect (7.4) on caries, while methimazole increased (9.8) caries and proteinase decreased (7.1) caries. Salivary studies also confirmed the dosage effect, suggesting that the concentration of substance used has an effect on caries response. Examination of the thyroids in animals receiving these compounds confirmed their goitrogenic effects, although only thiouracil produced changes in the submaxillary gland granular tubules.

196. HEREDITARY AND ENVIRONMENTAL FACTORS IN EXPERIMENTAL DENTAL CARIES.—*Sam Rosen, Carl A. Hoppert, and H. R. Hunt, Michigan State University, East Lansing.* Keyes showed that caries could be induced in animals by intraoral inocula-

tion of fecal material. This finding suggested that hereditary factors (genes) might be insignificant in the development of caries-resistant and caries-susceptible lines of rats. The phenotypes in these two lines of rats could have been produced by exposure of the newborn rat to the maternal fecal material. The degree of caries could then have been influenced by types and numbers of cariogenic flora contained within the maternal feces. In order to test this hypothesis, entire litters of caries-susceptible and caries-resistant rats were exchanged within 24 hours after birth; that is, caries-resistant rats were fostered by a caries-susceptible mother, and caries-susceptible rats were fostered by a caries-resistant mother. The subsequent litters born to these rats were used as controls. The rats were given the Hoppert-Webber-Canniff coarse-particle diet with weekly supplements of raw beef liver. Examinations for gross occlusal fissure lesions were made macroscopically every 2 weeks. Forty-five susceptible rats with resistant foster mothers developed caries at 64 days of age. Thirty-eight susceptible rats with natural mothers developed caries at 65 days of age. Not all the resistant rats with susceptible or natural mothers have developed caries to date. However, the results so far indicate that there is no difference in caries experience between these two groups of resistant rats, the average caries time being over 200 days for each group.

197. The Effect of Several Calcium Compounds on Rat Caries.—*Sangiem Limbasuta and Erling Johansen, Department of Dental Research, University of Rochester School of Medicine and Dentistry, Rochester, New York.* For this study 25-day-old male and female rats were littermated into one control and four experimental groups. The cariogenic diet used had the following composition: 35 per cent sucrose, 30 per cent whole powdered milk, 30 per cent whole-wheat flour, 4 per cent alfalfa, and 1 per cent sodium chloride. In the experimental groups 2 per cent of the whole-wheat flour was replaced by an equal amount of the calcium compound. The number of animals in each group was as follows: Group I: control 15♂, 14♀; Group II: 15♂, 16♀, calcium carbonate; Group III: 9♂, 16♀, calcium acetate; Group IV: 11♂, 15♀, calcium chloride anhydrous; Group V: 12♂, 16♀, calcium chloride dihydrate. On the basis of number of lesions, the calcium carbonate group showed a significant increase in caries while the calcium acetate group evidenced reduction in caries. However, a reduced food consumption in the latter group might have accounted for the decrease. The calcium chloride groups had higher caries incidence than did the control group.

198. Testing of Carbonyl-binding Compounds as Inhibitors of Experimental Caries.—*Harold V. Jordan, Robert J. Fitzgerald, and Norma D. Berger, National Institute of Dental Research, National Institutes of Health, Bethesda.* Previous work in this laboratory has shown that sodium metabisulfite incorporated into a coarse-particle corn, high-sugar diet (NIDR No. 585) causes a marked inhibition of occlusal fissure caries in Sprague-Dawley white rats. Since this compound is considered to inhibit glycolysis by its ability to trap carbonyl compounds, a second carbonyl-binding agent—dimedone (5,5-dimethyl-1,3-cyclohexanedione)—was also tested for possible anticarious activity in rats. In the present study 24 rats were fed 0.3 per cent sodium metabisulfite in the diet for 90 days. The caries score was 82 per cent lower than that of the control group (2.8 ± 0.7 versus 20.3 ± 1.9). Final mean weight of the experimental group was 7.8 per cent lower than the control. Incorporation of dimedone into diet 585 at a concentration of 0.4 per cent resulted in a significant reduction in the caries score (7.1 ± 0.9 versus 15.7 ± 1.8). Final mean weight of the dimedone group was 2.6 per cent lower than that of the control. Dimedone was tested for in vitro growth inhibition against a variety of oral organisms. A single strain of rat oral streptococcus (FA-1) was inhibited at 0.4 per cent, the highest concentration tested. Other strains of streptococci, lactobacilli, micrococci, gaffkyae, and candidae were only partially inhibited at this concentration. At a subinhibitory concentration (0.0014 per cent) dimedone did not affect lactic acid production

by streptococcus strain FA-1. Sodium metabisulfite was previously shown to inhibit lactic acid production by strain FA-1 at subinhibitory concentrations.

199. Evaluation of Glycolysis Inhibitors as Cariostatic Agents.—*John W. Hein and R. S. Manly, Tufts University School of Dental Medicine, Boston.* Approximately 150 chemicals were tried for compatibility in a cariogenic diet. These chemicals had been selected from a much larger number on the basis of their ability to inhibit glycolysis in salivary sediment. After the 150 chemicals had been mixed with the diet, only 25 were released in sufficient amounts, when the mixture was extracted with water, to cause 50 per cent inhibition of glycolysis. These chemicals were given preliminary toxicity tests by feeding each of them in a cariogenic diet to five animals for 1 month. Nearly all chemicals had to be tried at concentrations less than the desired 1 per cent, in order to permit weight gains of the experimental animals to equal those of the control animals. Caries studies have been completed on four chemicals: 1,3,6,8,tetracyclo (6,2,1,1^{36}) dodecane (I); α-bromo hydroxybutyric acid lactone (II); 1-chloro-1-nitro propane (III); and 2-cyclohexene-1-caproic acid (IV), at levels of 0.2, 0.3, and 1.0 per cent, respectively. None of the chemicals had any influence on the number of carious teeth. Chemical I caused signs of toxicity and death of six animals and produced a statistically significant reduction of the dental caries score (1 per cent level); II showed no evidence of influence on dental caries scores, and III caused a reduction in scores that were significant at the 5 per cent level; IV caused a decrease in caries score of about 50 per cent and the reduction was significant at the 5 per cent level.

200. (Not presented.)

201. The Rapid Assessment of Cariostatic Agents in Albino Hamsters.—*Paul H. Keyes, National Institute of Dental Research, National Institutes of Health, Bethesda.* Albino hamsters known to be infected with a cariogenic flora were placed on a caries-test diet when 20–21 days of age (*J. D. Res.*, **38**:525, 1959). After 10–14 days carious lesions were detected in second molars and to a lesser extent in first molars. This occurred before the third molars erupted. At this time the agent to be tested for caries inhibition was added to the food or drinking water. The cariostatic effectiveness of the agent was then determined by observing the degree of arrestation of caries in the first and second molars and the degree of prevention of caries in the third molars. Highly effective agents, e.g., erythromycin, caused a marked reduction in plaque within a few days. Decayed areas became intensely dark brown or black within 7–10 days, and the newly erupted third molars remained caries-free. In untreated control animals caries progressed at a rampant rate, causing extensive cavitation in all molar teeth within the total test period of 35–42 days.

202. Dietary Influences on Oral Flora and Caries in the NMRI-D Rat.—*C. A. Ostrom, Dental Division, Naval Medical Research Institute, Bethesda.* Two experiments were designed to study the frequency and types of organisms recoverable from oral samples of rats on experimental diets which would produce differences in caries incidence and severity. Control diet 30P4 had the following percentage composition: casein, 30.0; sucrose, 61.8; HMW salt mixture, 4.0; complete vitamin mixture, 2.2; linoleic acid, 1.0; and corn oil, 1.0. In the first experiment, diet 30P4 was compared to two variations: diet 30P4S contained corn starch substituted for the sucrose; and diet 30P4SF contained 53.8 per cent corn starch and 8 per cent corn oil substituted for the sucrose in the control diet. In the second experiment, diet 30P4 was compared with diet 30P4F, which contained 45.8 per cent casein, 4 per cent corn oil, and 12 per cent lard substituted for the sucrose, and Shaw's published diet No. 170. Weanling female rats in groups of 10 were maintained under standardized conditions in individual screen-bottomed cages with diet and water available ad libitum for 70 and 80 days, respectively. Mouth-swab samples were taken weekly in the first experiment and at 20-day intervals in the second experiment. Aliquots in 1 per cent

yeast extract cultured on Rogosa's SL agar, 5 per cent defibrinated sheep's blood azide agar, and 15 U/ml bacitracin nutrient agar were used to enumerate lactobacilli, streptococci, and coliforms. Progressive reduction in caries incidence and severity ranging from 40 to 65 per cent was observed in first, second, and third molars, with the exception of diet 30P4SF, which produced more caries than either diet 30P4S or30P4F. In the first experiment, the lactobacillus counts showed a downward trend on diet 30P4S, while on diet 30P4SF the counts stayed as high as the control diet. In the second experiment, differential counts showed that the homofermentive strains disappeared within 20 days on all diets, while the heterofermentive strains dropped in animals on diet 30P4F and the Shaw diet from a pre-experiment 1×10^4 to less than 100 per sample after 40 days. Differential counts for streptococci fluctuated widely. In general, the mean numbers of alpha and beta streptococci appeared gradually to increase about 1,000 fold during the 80-day period, but the gamma hemolytic streptococci were markedly reduced on all diets. The coliform counts fluctuated less widely and stayed at the same level throughout the experimental period.

203. Experimental Caries Inhibition by Intermittent Exposure to Penicillin.—*R. J. Fitzgerald, H. V. Jordan, and W. L. Poole, National Institute of Dental Research, National Institutes of Health, Bethesda.* Four groups of 24 weanling female Sprague-Dawley rats were placed on a coarse-particle corn, high-sugar diet 585. The animals in Group I received 20 units of penicillin per gram of diet one day per week, starting on the first day of experiment. Animals in Group II received the penicillin-containing diet the first of every 4 weeks. The remainder of the time, both groups received unsupplemented diet 585. A third group received the penicillin-containing diet for the full experimental period of 91 days. The fourth group received only unsupplemented diet 585. At the termination of the experiment the average caries experience per animal estimated by Cox's method was as shown in the table. The

Group	Regimen	Carious Teeth	Carious Surfaces	Caries Score
I.....	Penicillin 1 day/week	4.2	4.3	5.4
II.....	Penicillin 1 week of 4	4.9	5.1	6.7
III.....	Penicillin 91 days	3.7	3.9	3.9
IV.....	Control	7.2	8.7	13.9

values in each category were significantly less ($P < 0.01$) for the penicillin groups than for the untreated controls. The results indicate that it is possible to obtain marked inhibition of the development of caries in experimental animals by intermittent exposure to relatively low concentrations of penicillin.

204. Reduced Caries Activity in Offspring of Rats Receiving Tetracycline.—*I. Zipkin and Rachel H. Larson, National Institute of Dental Research, National Institutes of Health, Bethesda.* Sprague-Dawley rats were bred, and insemination was diagnosed by vaginal smear. The animals were then divided into four groups; A, B, and C received a solution containing 2.5 mg/ml of tetracycline hydrochloride; and D received distilled water. Group A ingested the antibiotic during gestation only; group B, during gestation and 14 days of lactation; and group C, during gestation, lactation, and the remainder of the preweaning period. Group D received distilled water until weaned at 21 days of age. Purina Lab Chow was available during gestation and for 14 days postpartum, and non-cariogenic diet 550 was offered thereafter until the pups were weaned. All pups then received distilled water and caries-test diet 636 for 60 days. The mothers receiving tetracycline showed marked fluorescence in the incisors but little, if any, in the molars. The progeny at weaning showed pronounced fluores-

cence of both the incisors and the molars. Tetracycline did not affect either fluid consumption in the mother or litter production. In the progeny, tetracycline administration did not interfere with fluid and diet intake or weight gain. The caries scores for the offspring of dams in groups A, B, C, and D were 2.3, 4.5, 1.8, and 20.3, respectively. It is apparent that caries activity was markedly reduced in offspring receiving tetracycline prior to weaning.

205. Effect of Tetracycline on Transmission of Dental Caries in Rats.—*Rachel H. Larson and I. Zipkin, National Institute of Dental Research, National Institutes of Health, Bethesda.* We have found that tetracycline consumed by mother rats during pregnancy and the first 2 weeks of lactation may be associated with reduced caries activity in the offspring. Two experiments were run to study the transmissible nature of caries activity in white rats. Experiment I consisted of the following groups: 34 control weanlings; 38 offspring of mothers given tetracycline as mentioned above; and a mixed group of 35 control weanlings and 35 offspring born of dams treated with tetracycline, one of each type per cage. During the test period the young rats were housed two per cage and fed diet 636 and distilled water for 60 days. The control animals showed extensive caries activity (25.1 carious buccolingual areas per rat); the tetracycline-treated animals showed a minimum of caries activity (6.8); and the animals in the mixed group showed extensive caries activity (controls = 19.4, tetracycline-treated = 19.3) whether or not their mothers had received treatment with tetracycline. Experiment II consisted of 4 groups of 24 weanling rats each, with variations in the drinking fluid only during the first week on the caries-test diet, as follows: the controls received distilled water; a second group received distilled water to which fresh fecal pellets collected from older control animals on diet 636 were added; a third group received 0.25 per cent tetracycline; and a fourth group 0.25 per cent tetracycline plus fecal pellets as above. The experimental conditions were the same as in Experiment I. The results showed extensive caries activity in the control group (34.9) and the group with feces added to their water (42.2), and only minimal activity in the tetracycline-treated groups (3.0 and 2.3, respectively). These experiments show that tetracycline treatment may be an effective means of reducing caries activity, but only if the rats are protected from a source of the transmissible factor after treatment with tetracycline.

206. Effects of Metaphosphoric Acid Supplementation on Morphology and Caries Incidence of Hamster Molars.—*Abraham E. Nizel, Robert S. Harris, and Janet Parker, Massachusetts Institute of Technology, Cambridge.* The golden Syrian hamster was used in two experiments in which M.I.T. diet No. 10 (whole corn meal, 47; sucrose, 14; non-fat milk, 20; plastic cream, 10; alfalfa, 6, liver concentrate N-F [1:20], 2; and sodium chloride, 1 per cent) was used as a caries-producing diet. The phosphorus level of this diet was 0.40 per cent in the first experiment and 0.38 per cent in the second. The phosphorus level was doubled (2P) and quadrupled (4P) by adding metaphosphoric acid, and these diets were fed to comparable groups of 20 hamsters. The animals received these diets from age 8 to 100 days in one experiment and up to 70 days in the other. In the latter experiment a more cariogenic diet suggested by Keyes was fed all animals for an additional 90 days. A binocular dissecting microscope was used for the dental examination. Every animal in the 4P group had a unique occlusal morphology. The molar teeth were flatter-cusped, shallower-fissured, and more lustrous than those in the control group and the 2P group. Practically no food retention was seen in occlusal surfaces of the molars of the 4P group, distinctly different from the controls. The caries incidence averaged 87 and 100 per cent in the two control groups and 0 and 9 per cent in the two groups fed the high-phosphorus diets.

XVIII. TRANSPLANTATION, IMPLANTATION, AND BONE STUDIES. MARCH 20, FORENOON, SECTION B

207. THE DEVELOPMENT OF THE POSTNATAL MOUSE MANDIBLE AS AN ISOLOGOUS SUBCUTANEOUS TRANSPLANT.—*Lloyd Henry Truax, Jr., Section of Dentistry and Oral Surgery, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.* Right and left halves of mandibles from 2-day-old mice were implanted under the skin of immature mice to compare their morphogenesis with the morphogenesis of the mandibles in normal mice. The two halves of each mandible were implanted into the dorsal-lumbar region of each of the 28 immature female hosts of the same strain (BALB/c). The controls consisted of 18 mice of the BALB/c strain. Four animals were sacrificed at the age of 2 days. At each interval of 5 days up to 42 days, another control was sacrificed and four implants removed; thereafter, at intervals of 10 days up to 102 days. With revascularization, osteogenesis progressed; growth or development of the dentition did not occur. Periosteal deposition occurred over the original surface of the implanted bone, while the condlyar and gonial cartilages wer prematurely and irregularly replaced by bone. After a period of accretion upon the initial form, resorption became an obvious characteristic, first observed after 20 days and observed in all specimens after 35 days of implantation. Halves of mandibles from 2-day-old mice implanted under the skin did not grow and develop in the same ways as did mandibles of the control mice; and, unlike implanted long bone, the implanted halves of mandibles did not attain approximately normal size and shape.

208. A STUDY OF METHODS OF STORAGE OF TOOTH TRANSPLANTS.—*E. R. Costich, J. K. Avery, and R. S. Mackenzie, University of Michigan School of Denistry, Ann Arbor.* This investigation was carried out to compare refrigeration and tissue culture as methods of storing teeth to be used later in transplantation procedures. The storage time as well as the method was studied. Short-rooted (young) as well as long-rooted (mature) molar teeth of Syrian hamsters were used. Second molars of animals of 16 and 45 days of age were removed with special instruments. One series was placed in tubes and cultured, and the other series was quick-frozen in dry ice and alcohol. The latter teeth were stored in a deep-freeze at —15° C. These teeth were brought to room temperature and planted in freshly prepared sites after storage times of 24 hours, 1 week, and 2 months. Cultured teeth were incubated for either 3 or 6 days before plantation. Hamsters were sacrificed at intervals of 1–99 days. Histologic findings for supporting tissues of long-rooted teeth were similar in all groups. Periodontal attachment was observed to occur first at the cervical area. By 14 days, connective tissue replaced the necrotic pulpal tissue. At 28 days, the new tissue in the pulp chamber was well established, containing blood vessels and nerve fibers. Some osteodentin or bone and no odontoblasts were observed. By 42 days, bone had filled many pulp chambers, and, in a few, functional bone marrow was observed. Most of the long-rooted teeth were noted to be in a good functional relationship. Ankylosis and external root resorption appeared frequently. The short-rooted teeth were lost or considered failures. This was probably due to a lack of stabilization at the time of replantation.

209. POSTOPERATIVE ANTIBIOTIC THERAPY USED IN ASSOCIATION WITH HAMSTER REPLANTATION PROCEDURES.—*Viola D. Flanagan and Hugh I. Myers, University of Kansas City School of Dentistry, Kansas City, Missouri.* In the past, one of the frustrating problems encountered in hamster replantation technics has been that of postoperative infection. Antibiotic therapy was therefore introduced in an effort to improve surgical management. A broad-spectrum antibiotic, small enough for an animal to tolerate, was desired. After testing several types, it was found that a tetracycline hydrochloride product* seemed to be most suitable. Dosage was determined from that established for the human (i.e., 2.5 mg/25 gm per animal) and was given by deep intramuscular injection for 3 consecutive days following surgery. The unilateral surgi-

* Panomycin, The Upjohn Co.

cal removal of lower left second molars was performed under nembutal anesthesia followed by immediate replantation. Postoperative diet consisted of fortified semisolid formula for 15 days, then unaltered Purina Laboratory Chow for 15 days. The animals were sacrificed by intracardiac injection of nembutal, and histologic sections were prepared for study. Evaluations were determined by pulp regrowth, attachment of peridental membrane, migration of epithelium, and amount of root resorption. Plus 1 and 2 were considered unsuccessful, and plus 3 and 4 as successful takes. There was an almost complete absence of tissue inflammation. Percentage of good takes was no less than 73 per cent, and one group yielded 100 per cent successful results.

210. Effects of Osseous Implant Materials on Regeneration of Alveolar Cortex.—*Philip J. Boyne and Harvey W. Lyon, Dental Division, Naval Medical Research Institute, National Naval Medical Center, Bethesda.*—Recent studies have demonstrated that clinical evidence of labial cortex regeneration in healing human alveolar bone defects is not necessarily reflected in the microscopy of the involved tissues. In order to observe the microscopic tissue changes involved in the postoperative osseous healing of areas of periapical bone destruction in man, eight clinical patients were selected having similar multiple areas of periapical radiolucency involving anterior teeth. Following root-canal therapy and periapical curettage, the area of labial cortex destruction was measured. One or more of the defects in each patient were implanted with an osseous material. No implant material was placed in the remaining periapical area. Boiled, defatted heterogeneous bone, ethylene diamine–treated bone, and freeze-dried homogeneous bone were used in this study. The operative sites were re-exposed, and biopsies taken in order to obtain histologic specimens, 4, 5, 6, and 8 months postoperatively. Complete regeneration of the labial cortex was found to have occurred 5 months after surgery in both implanted and control defects having a diameter of 8 mm. or less. Larger control areas exhibited limited cortex formation at 8 months, while implanted areas demonstrated marked cortex re-formation. The results of this study would suggest that periapical bone defects tended to heal with the early regeneration of alveolar cortex. The formation of fibrous connective tissue filling the cortical defect from the overlying soft tissues appeared to be influenced by the size of the defect.

211. Resorption Characteristics of Bone and Plaster of Paris.—*William H. Bell, University of Texas Dental Branch, Houston.* Previous experimental and clinical results suggest that the success of a bone graft is partially dependent on rapid resorption of the graft by the host. The results of these studies indicated that autogenous bone implants were resorbed more quickly than anorganic bone or freeze-dried bone. The following study was carried out to compare the rate of resorption of implants of autogenous bone, freeze-dried bone, homologous bone, anorganic bone, bovine bone, and plaster of Paris. The implants, which were 7 mm. in diameter and 2 mm. in thickness, were placed intermuscularly between the heads of the gastrocnemius muscles of seventeen adult mongrel dogs. The resorption rates of the implants were determined by radiographs taken at weekly intervals. Plaster of Paris implants were the quickest to be resorbed, taking an average of 4.7 weeks. Resorption of autogenous implants required an average of 7.0 weeks; homologous, 10 weeks; and bovine bone, 11.5 weeks. Anorganic and freeze-dried bone resorbed slowly, frequently showing minimal or no radiographic change after 16 months.

212. A Method for Estimating the Rate of Bone Resorption.—*Harold Wolsztyniak and Henry Jeffay, College of Dentistry and College of Medicine, University of Illinois, Chicago.* Weanling rats were placed on a diet containing radioactive calcium until the specific activity of the bone approximated the specific activity of the blood (3 weeks). The animals were then placed on a non-radioactive calcium diet and sacrificed serially during the next 45 days. The changes in the specific activity of the bones (femur shaft, epiphysis, and whole bone and mandible, rib, skull, and humerus), teeth (incisor and molar), blood, urine, and feces were followed. The

decrease in the specific activity of calcified structures was established as a function of at least three factors: (1) increase in bone calcium (growth), (2) physiochemical exchange, and (3) resorption. The contribution of each of the factors leading to the observed changes in the specific activity of the bones was calculated. It was concluded that during the rapid growth phase either bone resorption did not occur at a measurable rate or that all resorbed calcium was translocated in the same bone. There was a measurable rate of bone resorption in those animals which weighed more than 300 gm. at the time of sacrifice.

213. Osteogenesis and Repair of Acellular Teleost Bone.*—*Melvin L. Moss, Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York.*—Many higher teleost fish have bony endoskeletons totally devoid of osteocytes. In this preliminary study, comparative data demonstrated the differences between osteogenesis and repair in cellular and acellular fish bone. Concerning appositional bone growth, in acellular bone there was an unusual periosteum within which the osteogenic cells gradually hypertrophied. With the onset of matrix formation and calcification, osteoblastic (osteocytic?) necrosis occurred, followed by total disappearance of lacunae. In cellular bone a more typically mammalian type of periosteum was found. In both forms a resting periosteum was extremely difficult to demonstrate. Endochondral bone formation was apparently similar in both skeletal types. Direct metaplasia of a cartilage-like tissue into bone, both cellular and acellular, was frequently observed. Fracture of mandibular and opercular skeletal elements, both osseous and cartilagenous, was followed by markedly different responses dependent on the bone type. Cellular bone skeletal fracture was followed by intense connective tissue, periosteal, and perichondral reactions, essentially similar to the mammalian response. Callus formation was extensive, and skeletal continuity was quickly reestablished. Direct metaplasia of callus tissue to both osseous and cartilagenous tissue was frequent. Identical trauma to an acellular bone skeleton was followed by the most sparse tissue reactions and by only indifferent attempts at osseous repair.

214. Succinic Dehydrogenase in Osteoclasts in Resorbing-Bone Tissue Cultures.—*Paul Goldhaber, Harvard School of Dental Medicine, Boston, and Russell J. Barrnett, Yale University School of Medicine, New Haven, Connecticut.* The experimental production of bone resorption initiated by introducing high concentrations of oxygen into the gas phase of young mouse calvaria in tissue culture provides a versatile system for further study of this aspect of bone physiology. The presence of osteoclasts and their aggressive action against the resorbing-bone surface (as visualized by time-lapse microcinematography) has been reported previously. The present report is concerned with the histochemical localization of succinic dehydrogenase in resorbing-bone cultures. An incubating medium for the histochemical demonstration of succinic dehydrogenase was made up, containing phosphate buffer, activators, sodium succinate as substrate, and a tetrazolium salt, Nitro BT, as electron acceptor. Addition of 0.5 ml. of this solution to the supernatant medium of 3–5-day resorbing-bone cultures without prior fixation resulted in the gradual development of an intense bluish-violet coloration. indicative of high enzyme activity, primarily within giant cells (presumably osteoclasts) adjacent to resorbing-bone surfaces and scattered throughout the resorbed area. Similar enzyme activity was also observed in apparently inactive osteoclasts located in regions where bone resorption was minimal. Time-lapse microcinematography studies prior to and during histochemical staining of the same osteoclasts clearly demonstrated succinic dehydrogenase activity within these bone-resorbing cells. In view of the intimate association of this enzyme with the mitochondria, it is likely that osteoclasts have a high metabolic activity, thereby supporting the interpretation of previous cinematographic evidence concerning the active role of osteoclasts in the process of bone resorption.

* This investigation was supported in part by Research Grant A-1930-C from the N.I.H., U.S. Public Health Service.

215. Factors Which Influence Osteolathyrism in the Periodontium.—*Irving Glickman, Tufts University, Boston, and Hans Selye, University of Montreal.* A histologic study was conducted to determine the effect of specific agents upon the response of the periodontium to an osteolathyrogen such as aminoacetonitrile. Sixty-eight female Sprague-Dawley rats, 100 gm. average initial body weight, were divided into seven groups as follows: aminoacetonitrile, 10 mg daily, administered subcutaneously in 0.2 ml. H_2O in two 5-mg. doses; AAN plus thyroparathyroidectomy; AAN plus thyroparathyroidectomy plus thyroxin (1 μg. daily); AAN plus thyroxin (500 mg daily); AAN plus partial hepatectomies; AAN plus ACTH (10 I.U. daily); and a control group. After a 20-day experimental period the AAN-induced changes in the periodontium included osteoporosis of alveolar bone with thinning and irregularity of trabeculae, enlarged marrow spaces, reduction in bone apposition adjacent to the periodontal membrane and along endosteal surfaces, and increased osteoclasis. Alterations in the periodontal membrane varied from reduction in cellularity and fiber content to marked increase in fiber density with irregular hyalinization. In scattered areas of the periodontal membrane there was pronounced bone-formative activity, with apposition along adjacent bone trabeculae. Cementum deposition was reduced. The lathyric manifestations in the periodontium were accentuated by partial hepatectomies, thyroparathyroidectomy, and thyroparathyroidectomy plus thyroxin (1 μg. daily), reduced by ACTH and almost completely nullified by thyroxin in large doses.

216. A Microradiographic Study of Compact Bone.—*M. B. Quigley and E. Smith, University of Alabama Medical Center, Birmingham.* The Haversian system or osteone is accepted as the basic structure of compact bone. In this study the concentric lamellae which make up such an osteone were found to be dissimilar in some aspects. The intra-Haversian junction line demonstrated in some texts appeared to be a hypercalcified lamella. The lamellae adjacent to the Haversian canal were or were not more calcified than the deeper (intra-Haversian) lamellae. In attempts to increase contrast in the microradiographs by the absorption of heavy metals, the various lamellae reacted differently. In some sections the absorption of heavy metals resulted in microradiographs which resembled the "Maltese cross" appearance of bone as seen in polarized light. Cement lines surrounding osteones appeared as radiopaque zones. Some Haversian systems, which were radiolucent in microradiographs of untreated sections, became more radiopaque than surrounding tissue after exposure to solutions of heavy metals, suggesting increased reactivity of these systems to the solution. The zones adjacent to the lacunae were more radiopaque and were therefore, considered more highly calcified than the adjoining bone. This probably accounted for the resistance to chemical reagents previously noted by some authorities.

217. Bone Changes Following Surgical Intervention: The *Macaca mulatta rhesus* Skull.*—*John A. Cameron and George S. Kendrick, Department of Anatomy, Baylor University College of Dentistry, Dallas.* Six animals were operated and sacrificed from December, 1956, to August, 1959. Two animals received unilateral operations for removal of bone along the posterior superior ramus, including the posterior surface of the condyle. Observation of the dried skull revealed no cranial asymmetry. The defect was repaired on one operated side by apposition of new bone with no resultant mandibular asymmetry. The other animal demonstrated little or no asymmetry of the skull. Removal of not over one-fifth of the posterior and superior ramal surface seemed to have little or no effect on the ultimate shape of the mandible. Two animals had condyles removed and replaced with epiphyseal grafts. The distal head of the fifth metatarsal, including at least 2 mm. of the bony diaphysis, was ligated to the mandibular neck. The condylar growth center was replaced with a metatarsal graft which included growth centers of the epiphysis and the epiphyseal plate. Observation of the dried skulls revealed that one graft remained in the glenoid fossae.

* This work was supported by Research Grant D-246 from the N.I.D.R., U.S. Public Health Service.

There appeared to be some enlargement of the bony epiphysis. Since there was no osseous union, the epiphyseal plate was replaced with dense fibrous connective tissue. There was a remarkable lack of cranial asymmetry. The graft in the second animal was displaced in a medial and inferior direction. Two animals had their condyles removed. One received no graft. Cranial, facial, and mandibular asymmetry was observed. The other animal received a metatarsal graft almost a year following condyle removal. This animal demonstrated no growth from the metatarsal graft.

218. Alveolar Bone Loss in Mongolism.—*Richard A. Winer, M. Michael Cohen, and Stanley M. Schwartz, Tufts University School of Dental Medicine, Boston.* Roentgenographic examination of 100 Mongoloid institutionalized patients between the ages of three and thirty revealed alveolar bone loss ranging from moderate to severe. The alveolar bone loss in the Mongoloid group was compared roentgenographically with a similar group of non-Mongoloid patients in the same institution and a comparable group of patients having had complete radiographs taken in a dental-school clinic. In assessing the degree of bone loss from the radiograph the following criteria were used: 0—no observed bone loss, 1—loss of crestal bone, 2—one-quarter bone loss, 3—one-half bone loss, and 4—three-quarters or more bone loss. Bone loss was observed in the primary, mixed, and permanent dentitions. Approximately 96 per cent of the Mongoloid patients manifested varying degrees of bone loss. The most frequent sites were the maxillary and mandibular anterior regions. In the non-Mongoloid, mentally subnormal patients, alveolar bone loss was observed radiographically in approximately 40 per cent of the cases. Only 6 per cent of the patients from the dental clinic showed alveolar bone loss.

219. A Roentgenographic Study of the Trabecular Pattern of the Alveolar Processes of the Human Maxilla and Mandible.—*Nicholas J. Brescia, Loyola University, Chicago.* A group of sixty-four male individuals (twenty to thirty years of age with full dentition) was examined roentgenologically in order to ascertain the possible types of distribution and arrangement of spongiosa in the alveolar processes of the maxilla and mandible. In the living as well as in the sample of skulls, two distinct patterns of the architecture of the spongiosa were found. The first type showed a regular horizontal arrangement of the spongy trabecular running from the walls of the socket of one tooth or root to the next. The second type showed an arrangement of spongiosa trabeculae that was irregular but was characterized by the much greater number of trabeculae in any specific area of the alveolar process. Type I, as well as Type II, showed a variation in thickness of trabeculae and size of marrow spaces. This variability allowed the division of each type into two subtypes; however, there were some individuals in whom the division into subtypes was difficult, if not arbitrary. The presence of two rather widely different patterns in the spongiosal architecture of the alveolar processes necessitates great caution in the diagnosis of skeletal pathologies from intraoral dental roentgenograms.

XIX. SALIVARY GLANDS. MARCH 20, FORENOON, SECTION C

220. DNA Synthesis of Mouse Submandibular Gland.—*Mario Santangelo, Loyola University School of Dentistry, Chicago.* A determination of DNA synthesis of the cells of the submandibular gland of the C_{57} black $\times$ albino mouse was studied. Eighteen mice, 300 days of age, were injected intraperitoneally with 0.05 cc. of tritiated thymidine with a total activity of 50 microcuries. The mice were sacrificed after 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 12 hours, 24 hours, and 48 hours. The grossly dissected submandibular gland was fixed in a 10 per cent neutral formalin solution, imbedded in paraffin, sectioned at 3 μ, deparaffinized, and stained by the Feulgen technic for DNA. The stained sections were autoradiographed by the stripping method, using stripping emulsion,* and the processed emulsion ex-

* Kodak AR-10.

amined under a microscope. Five thousand cells were counted under oil immersion at each of the sacrifice periods, and the number of radioactive nuclei per 5,000 cells were recorded as percentages. In the first hour those cells undergoing DNA synthesis became labeled. From 2 to 4 hours, the slight rise might be attributed to a continuation of the synthesis of just the beginning of mitosis. Between 4 and 12 hours, mitosis occurred, and from 12 and 48 hours a plateau appeared to be established. This might be interpreted as an extremely long interphase period before mitosis recurred. At no time were more than 1 per cent of the total cells counted labeled. It was estimated that the turnover rate of the submandibular gland of the mouse is extremely low.

221. Characteristics of a Mucoid from the Bovine Submaxillary Gland.—*F. Draus and S. Wah Leung, University of Pittsburgh School of Dentistry.* Aqueous extracts of bovine submaxillary gland, after deproteinization with chloroform and Naxonate G followed by dialysis and lyophilization, yielded a mucoid which was readily soluble in water. The mucoid showed the following analysis: ash, 2.75 per cent; Kjeldahl N, 10.0 per cent; sialic acid, 25.0 per cent; glucosamine, 20.1 per cent; and protein, 6.8 per cent. This protein could not be removed by repeated deproteinization. The indicated components did not make up the total composition, since all the nitrogen was not accounted for. The compound exhibited the typical absorption in the ultraviolet region, with a maximum absorption of 217 μ. A potentiometric titration indicated a pK of 5.80. A determination of the optical rotation showed that the mucoid exhibited a specific rotation of $+5.2°$. Paper chromatography of the carbohydrate fraction indicated the presence, in addition to the sialic acid and glucosamine, of a third component as yet unidentified, with an Rf of 0.183 (butanol:acetic acid:water). Chromatography for protein indicated the presence of at least 14 amino acids.

222. Effect of Bovine Submaxillary Gland Extract on Ultrafiltration of Calcium.—*R. Farquhar, S. Wah Leung, and F. Draus, University of Pittsburgh School of Dentistry.* The effect of a submaxillary gland extract on the diffusion of calcium across a semipermeable membrane was studied by employing the Toribara ultrafiltration chamber. Test solutions were prepared by adding various concentrations of submaxillary gland extract to calcium solutions buffered at different pH's. Extract-free calcium solutions served as controls. Diffusibility of the calcium was determined by comparing the calcium concentration of the original solution with that of the ultrafiltrate, following ultrafiltration in the Toribara apparatus at 1600 rpm for 6 hours at 4° C. Calcium was determined by flame photometry. In the absence of the bovine extract no difference was found in the calcium concentration of the ultrafiltrate and that of the original solution; i.e., the calcium was freely diffusible. When extract was added to a level of 0.1 per cent, the calcium concentration of the ultrafiltrate at pH's 8 and 10 was 11–15 per cent less than in the original. No differences were found at pH 6 or lower. The results indicated that the presence of the bovine extract decreased the diffusible calcium. The relationship between this phenomenon and pH, extract concentration, and calcium concentration was reported.

223. Bovine Submaxillary and Sublingual Mucins.—*Ward Pigman, Shigeru Tsuiki, and Y. Hashimoto, University of Alabama Medical Center, Birmingham.* Mucins were isolated by mild procedures from aqueous extracts of ground bovine submaxillary and sublingual glands. The sublingual mucin was obtained by successive precipitations at 2°–5° with ammonium sulfate (at 10–20 per cent saturation), sodium sulfate (2.3–6.5 per cent), and 5 per cent calcium acetate and 60 per cent ethanol. The submaxillary mucin was obtained by electrodeposition and subsequent ammonium sulfate fractionation. Physical methods, especially visometric measurements, indicated that no appreciable degradation occurred during the preparations. The products dissolved readily to give clear, viscous, stringy solutions. Bovine submaxillary mucin contained about one-third peptides, and the remainder was composed of equal molar ratios of sialic acid and galactosamine. Small amounts of glucosamine,

L-fucose, and D-galactose were also present, apparently as impurities. Bovine sublingual mucin generally had a composition much like known blood-group substances. The best product, probably still not pure, contained less than 20 per cent of protein. The carbohydrate portion was composed of the following molar ratios of sugar units: D-galactose, 2; hexosamine (galactosamine and glucosamine), 2; sialic acid, 1; and L-fucose, 1. The purified mucins did not give mucin clots upon acidification with weak acids unless serum albumin was added prior to the acidification.

224. PRELIMINARY STUDIES OF THE VASODEPRESSOR ACTIVITY OF BOVINE SUBMAXILLARY GLAND EXTRACT.—*B. Cohen, J. Jumber, G. Eden, and S. Wah Leung, University of Pittsburgh School of Dentistry.* The presence of a vasodepressor substance in saliva has been reported by numerous authors. These observations have been confirmed in our laboratory. In addition, we isolated from the submaxillary glands of freshly slaughtered cattle a substance having similar blood pressure–lowering effects when injected intravenously into the cat. The crude material was obtained by homogenizing the glands with distilled water, filtering through Celite, and lyophilizing the filtrate. The resulting white, fluffy, water-soluble powder contained the vasodepressor principal. Fractionation of the redissolved material with graded percentages of ammonium sulfate showed the principle to be almost completely precipitated between 55 and 70 per cent ammonium sulfate. Using the cat as the assay animal, the active principle was found to be heat-stable, non-dialyzable, not affected by pH's between 3 and 6.5, and unlikely to be acetylcholine or histamine.

225. SALIVARY GLANDS AS ENDOCRINE ORGANS.—*Z. Z. Godlowski and J. C. Calandra, Departments of Pathology and Medical Diagnosis, Northwestern University Dental School, Chicago.* Total bilateral extirpation of submaxillary and sublingual glands produced in 5 dogs a significant increase in insulin sensitivity as measured by an insulin-sensitivity test. The glucose-tolerance test in the same animals showed a less significant and less constant increase in the utilization of the extrinsic blood glucose. Similar results were obtained in 4 dogs with complete bilateral ligation of the secretory ducts, which resulted in the atrophy of the submaxillary and sublingual glands. Fourteen control experiments failed to show any significant fluctuations in insulin sensitivity or in utilization of the extrinsic blood glucose. It was concluded that submaxillary and possibly sublingual glands were producing a factor which, if removed, potentiated the action of insulin; this factor was called "submaxillary insulin inhibitor" (S.I.I.).

226. EXPERIMENTAL ENLARGEMENT OF THE SUBMANDIBULAR SALIVARY GLANDS.—*Herbert Wells, Biological Research Laboratories, Harvard School of Dental Medicine.* As reported previously, a rapid and marked enlargement of the submandibular salivary glands of rats follows amputation of the lower incisor teeth. Our previous studies suggested the hypothesis that there are receptors located within the incisor teeth which, when stimulated by amputation, initiate a chain of events, primarily neural, which leads to enlargement of the submandibular glands. As part of a continued analysis of this problem, pharmacologic and surgical technics were utilized. There appeared to be a barbiturate-sensitive step in the process, since pretreatment with barbital completely blocked the response to amputation. Complete blockade was also achieved by pretreatment with the ganglionic blocking agent, chlorisondamine chloride.* Surgical exposure of a portion of the incisor pulp chamber in the mandibular ramus and injection of alcohol or formalin into the pulp had no significant effect, indicating that transmission through the pulp was not essential. The effective stimulus was apparently not carried over the inferior alveolar nerve either, since section of a large portion of this nerve had no significant blocking effect. An important pathway appeared to travel through the pterygomandibular space, however, since injection of alcohol into this area completely blocked the response. The potent adrenergic blocking agent, Dibenamine, not only completely blocked the response to amputation but

* Ecolid, Ciba Pharmaceutical Products, Inc.

caused a profound atrophy of the glands, indicating that the sympathetic nervous system may play an important role not only in the response to incisor amputation but in the normal regulation of the size of the submandibular glands as well.

227. Effect of Parotin on Salivary Gland and Pancreas in Mice.—*Harold S. Fleming, Howard University, Washington, D.C.* Parotin, an extract of bovine parotid glands, is known to affect certain organs and systems of the body. Rockland mice receiving 0.30-mg. injections of this substance on alternate days showed significant changes in the salivary gland and pancreas. These changes were marked after five injections had been given. By ten injections, there were increases in vascular activity in both the salivary glands and the pancreas. This was striking about the duct systems of these glands, for associated vessels appeared larger and were well filled with red blood cells. Ductal epithelium in the salivary glands was proliferative, lumens were smaller but patent, and there was an increase in the amount of cytoplasm in relation to the nuclei. In the islets of the pancreas there was a proliferation of cells associated with the duct systems, an increase in their size, more small vessels, and proportionately a greater number of beta cells. Both the exocrine cells of the pancreas and the acinar groups of cells in the salivary glands underwent hyperplasia, and, in addition, there was increased mitotic activity. At times small vacuoles were noted in the cells of the exocrine pancreas, and fibrillar-like structures were evident in the cytoplasm of some of the larger cells. Interseptal spaces and fatty connective tissue were decreased in both glands. These studies further substantiate the wide-reaching effects of parotin in experimental animals.

228. The Isolation of Submaxillary Gland Acini and Ducts.—*Rose Mary Schneider and Philip Person, Veterans Administration Hospital, Brooklyn.* A method was presented for the separation of intact acini and of segmented ducts from rabbit submaxillary glands. It combines the principles of differential homogenization and differential centrifugation and introduces a new approach to tissue fractionation by permitting the initial recovery of anatomical cell aggregates amenable to further fractionation. Seventy per cent glycerol was used throughout as the suspending and wash medium. Acini were released by expressing the glands through bolting cloth, the "expressate" was concentrated at *ca.* $1{,}500 \times$ G, and the sediment washed several times by layering over the suspending medium and centrifuging at lower acceleration. The final sediment showed intact acini of the "special serous" type characteristic of rabbit submaxillaries. The duct fraction was prepared from the residue retained on the bolting cloth through which the acini were previously expressed. This residue was homogenized at high speed, the homogenate concentrated at $1{,}500 \times$ G, and the sediment washed by layering and centrifuging at progressively lower accelerations, below that employed for acini. The final sediment showed segments of ducts, predominantly of the striated or intralobular type. Negligibly low cytochrome oxidase values obtained for acini and for ducts attested to the relative intactness of these isolated aggregates and to their relative freedom from contaminating oxidative particulates, i.e., mitochondria. Disintegration of the intact structures at respective homogenizing speeds required for acini and for ducts resulted in striking increases in cytochrome oxidase values, due to the release of active intracellular mitochondria.

229. Transport in the Acinar Duct System of the Salivary Gland.—*Basil L. Henriques, Tufts University School of Dental Medicine, Boston.* A time-course study from t_o, the destruction of duct cells by $HgCl_2$, and the stop-flow technic of Malvin, Sullivan, and Wilde (*Physiologist*, **1**:58, 1957) were employed to investigate the localization of sodium, potassium, and water transport in specific areas of the acinar duct system of the submandibular gland of the dog. It was observed that potassium was secreted in the acini at concentrations about twice that of blood and that potassium in saliva was concentrated in the ducts at low flow rates. This was due partly to water resorption, as well as to active transport into the duct system. Sodium

was secreted in the acini at concentrations approaching those found in blood. About half of this was resorbed in the duct system at average flow rates, and the percentage increased to about 98 per cent at low flow rates. Conversely, the saliva was observed to increase in sodium concentration after standing for 2 minutes in the ducts. Because sodium and potassium were increased in their concentrations at very low flow rates and since water followed sodium through membranes, it was deduced that water was in part secreted into the saliva across the duct cells. Duct transport of water into saliva was corroborated by the fact that when the transductal transport mechanism of the ducts was destroyed, the flow rate was significantly reduced.

230. The Comparative Histology of Salivary Glands of Monkeys and Humans.—*Richard E. Barb and James A. Green, Department of Anatomy, Indiana University Schools of Dentistry and Medicine, Indianapolis.* Histologic comparisons were made of the three major salivary glands of the human and two species of macaques (*M. rhesus* and *M. cynomolgus*). The glands were fixed in Bouin's, Carnoy's, Helly's, and Luft's buffered acriline solutions. Paraffin sections were made and stained with a variety of stains. Monkey glands were more compact than the human glands, with little, if any, fat present within the former, while an abundance of fat was seen within the human parotid and submaxillary glands. Lobules were more apparent in the monkey glands. No sexual dimorphism was observed in the glands. The parotid glands were found to be purely serous in each of the species. The parotids of the two species of monkeys were structurally similar with respect to compactness, stainability of the component parts, and over-all structural plan. However, duct-cell heights in the three species varied; the rhesus excretory, secretory, and intercalated ducts had shorter epithelial cells than did the cynomolgus and human gland ducts. The rhesus and cynomolgus and submaxillary glands had more mucous components than did the human. The duct-cell heights varied in the different species. The sublingual glands of the three species appeared similar in structure and stainability. The rhesus and cynomolgus sublingual glands possessed some irregularly placed serous areas. These were not seen in the human. The monkey sublingual glands possessed some areas which were purely mucous, with no serous demilunes. This arrangement was not seen in the human. The parenchyma of the salivary gland of the human and the two macaques was similar in most respects.

231. Microcinematographic Observations on Giant-Cell Formation in Mouse Salivary Gland Cultures.—*Nicholas G. Grand, University of Illinois College of Dentistry, Chicago.* Fragments of salivary glands of 1–3-day-old mice were explanted in tissue culture. After several days of incubation at 37° C., giant cells appeared amid the fibroblast-like population of cells that had migrated from the explants and proliferated. The giant cells varied in size from 30 to 85 μ in diameter, had between one and four nuclei, and contained within their cytoplasm large granular masses located in the center of each cell. Time-lapse photographs, made with the use of a phase-contrast microscope, demonstrated the formation of a multinucleated cell, the formation and action of microfibrils and other cytoplasmic processes, and macrophage-like behavior of giant cells.

XX. ENDODONTIA AND HIGH-SPEED INSTRUMENTS. MARCH 20, AFTERNOON, SECTION A

232. The Endogenous Respiratory Quotient of Bovine Dental Pulp.—*Christian Schwabe and Alton K. Fisher, College of Dentistry, State University of Iowa, Iowa City.* Previous studies of respiration in bovine dental pulp showed survival in vitro for as long as 26 hours without exogenous nutritive support. This raised the question of whether its energy is derived from free or loosely bound carbohydrate in the connective tissue matrix or from its protein and lipid constituents. QO_2 and QCO_2 determinations were made for alternate 30-minute periods on the same tissue samples by use of Stanley-Tracewell R.Q. flasks supplied with Krebs-Ringer-bicarbonate sus-

pending fluid and Pardee's diethanolamine buffer as the CO_2 absorbent. R.Q. determinations were made on 12 embryonic, 12 young, and 5 mature samples of bovine dental pulp, classified on the basis of dentinogenetic activity. Determinations were begun 2½ hours after removal of teeth from the animal and were continued in most instances for 11 hours thereafter. The R.Q. 0.86 calculated for embryonic pulp suggested that carbohydrate was already exhausted by the time the first measurements of this active tissue were completed. Less active young pulp showed an R.Q. of 0.94, characteristic of carbohydrate metabolism at the end of the first period of measurements. Subsequent determinations showed the R.Q. to be declining through the protein and lipid ranges, stabilizing around R.Q. 0.7. Relatively quiescent mature pulp showed characteristic carbohydrate metabolism through four complete cycles of measurements and then began to consume its proteins and lipids. It also showed indirect evidence of glycolytic activity while glucose was still present in the tissue. It was concluded that carbohydrate reserves in dental pulp are limited and rapidly depleted, depending upon the activity of the tissue.

233. Reaction of the Pulp of Scorbutic Guinea Pigs to Injury.—*Clive I. Mohammed and Dorothy Mardfin, School of Dentistry, University of Puerto Rico.*—This study was based on complete serial sections through the mandibular incisors of 50 guinea pigs. Ten animals were fed a basal vitamin C–free diet. Ten animals were fed the basal diet, in addition to which they received intraorally a daily dose of 0.2 mg. of vitamin C. In all cases, pair-fed controls receiving 3 mg. of vitamin C daily were maintained. An additional 10 animals which also received 3 mg. of vitamin C daily ad libitum served as controls. Two weeks after the beginning of the experiment, cavities were prepared in the mandibular right incisors. All cavities were filled with a mixture of zinc oxide and eugenol. The animals were sacrificed at intervals varying from 12 hours after the operation to 5 days. Areas of the pulp underlying the cavities showed an increased amount of dentin formation. The type and amount of dentin varied between groups. There were no essential differences between the incisors of the pair-fed and ad libitum controls. In the experimental animals, the cells of the odontoblastic layer had lost their typical columnar shape and were low cuboidal. In animals receiving no vitamin C, there was an absence of dentin formation in the unoperated incisors. In the operated incisors, however, there was osteodentin formation around the entire circumference of the pulp. This would indicate that, although the cells had lost the typical morphology of odontoblasts, their potentiality to produce dentin under a stimulus was retained.

234. Pulpal Response in Rat Molars to Cavity Cutting and Fluoride-containing Acrylics.—*George W. Burke, Jr., Medical College of Virginia, Richmond.* This study consisted of a histopathologic evaluation of pulpal response to cavity-cutting procedures and the use of various fluoride combinations added to acrylic resins. The maxillary first molars of 176 white rats of a Wistar strain, approximately 3 months of age, were included in this study. Cavities were prepared on mesial surfaces of right and left molars, utilizing small burs operated at slow speed. Cavity preparations in 120 right first molars were restored with acrylic resins containing 2 per cent of various fluoride salts. The remaining 56 right molars were restored with acrylic resins free of fluorides and thus served as controls. All the left first molars were restored with zinc phosphate cement as further controls. Animals were sacrificed following restoration of cavity preparations on the third day and at various intervals through 28 days. Whole maxillae were prepared for sectioning at approximately 10 μ and stained with hematoxylin and eosin. Based on histopathologic studies, 87 per cent of pulpal reactions under fluoride-containing acrylics were classified as mild or moderate, while 13 per cent were classified as severe, including 1.3 per cent necrotic reactions. There was little or no difference between these reactions and those observed under control restorations.

235. Pulp Lability and Repair. IV. Pulpolytic Reactions.*—*Leo Zach, New York University College of Dentistry, and Gerson Cohen, Veterans Administration Hospital, New York.* In the course of studies on pulp lability and repair, the isolation of the physical variables incident to cavity preparation and their implication in pulpal response have led to a pointing-up of certain cellular and ground substance reactions. Transection of dentin led to a preliminary lysis of predentin in tubular continuity with the cavity. This was most evident in 1-week postpreparation specimens. Odontoblasts severely stressed were dominant. Edema and microvacuolization occurred under the pulpodental membrane and initiated a dissolution of predentin undecalcified ground substance. In their organizing capacity, disrupted odontoblasts adjacent to cut areas began to re-form, after 1-week "stress" predentin. "Scar" dentin, with its sparse tubular components, was formed first in the relative absence of odontoblasts and only later, as new odontoblasts differentiated, assumed a pattern of regular tubular dispersion. The "resting" odontoblast, elsewhere in the pulp, demonstrated a richer cytoplasmic structure; the predentin was thin and unstimulated. Cutting at speeds over 5,000 rpm without coolant within 2 mm. of pulp in rhesus adult teeth produced an invariable "burn" reaction characterized by intra-odontoblastic fluid collection, which distorted and later lysed these cells. The cell-free zone of Weil after 1 week showed marked fibroblastic activity. Resolution of cellular debris was effected by histiocytes. New endothelial buds proliferated into the reorganizing stroma. Shy of frank abscess formation, the early intrapulpal responses were marked by a freedom from the classic leukocytic calling-forth of acute inflammatory cells.

236. (Not presented.)

237. The Relationship of Pulp Response to Pulp Status at Treatment.—*Donald R. Porter, Norman H. Rickles, and Franklin D. R. Piacentini, University of Oregon Dental School, Portland.* This study was undertaken for the purpose of determining the relationship between response to vital pulpotomy therapy and status of the exposed pulp. At the time of pulp exposure the pulp of the isolated tooth was unroofed, and a section of pulp was amputated and placed in 10 per cent formalin. A culture was obtained from the pulp and placed in a thioglycolate tube and incubated. The tooth was then treated with $Ca(OH)_2$ in the vital pulpotomy procedure. The cultures were plated on blood agar, and antibiotic-sensitivity tests were performed. The tissue sections were examined microscopically after hematoxylin and eosin and Brown and Breen (modified Gram stain for tissue) staining. Patients who had received treatment were recalled after 12 months. Radiographs were taken, and evaluation of the treated teeth was done to establish success or failure of the treatment. Classification as success was contingent upon the absence of radiographic evidence of pathology and lack of clinical symptoms. Tissue sections from teeth with similar apparent prognosis for healing, i.e., absence of severe clinical symptoms and of pathological change on the pretreatment radiographs and evaluation, showed wide variations. Only a few sections showed absence of inflammation. Complexity of the pulp tissue ranged from acute pulpitis with cellular infiltration to chronic pulpitis with localized areas of necrosis. The Brown and Breen modified Gram stain permitted visualization of bacteria in many of the tissues sections and colonies in some. Contingency tables were utilized to test trends in a small sample of 12-month success and failure teeth. The variables were treated independently, and the chi-square test was applied at the 0.05 level. The presence of culturable bacteria was not significant in relation to success or failure. The state of inflammation—chronic pulpitis contrasted to subacute, acute, and absence of inflammation—was significant at the 0.01 level.

238. A Method for Obtaining Rat Molar Pulp Contents Aseptically.—*Lucile S. Smith and Gerald D. Tappe, College of Physicians and Surgeons, School of*

* This investigation was supported by Research Grant D-530 (C-2) from the N.I.D.R., U.S. Public Health Service.

Dentistry, San Francisco. A method for obtaining rat molar pulp contents aseptically was developed to enable study of infection in pulp canals. Rats were anesthetized with intraperitoneal injections of nembutal and demerol, then held immobile with the mouth open by means of a special animal operating tray. The maxillary molars were exposed through two 1-cm. parallel slits about ¼ inch apart cut near the center of a 5 × 7-inch rubber dam. The dam was held in place by anterior and posterior ligatures. The teeth were isolated with interproximal ligatures. The ligatures were passed through the cheeks and anchored by passing them around the board and tying the ends. The exposed molars and adjacent rubber dam were swabbed with 5 per cent phenol and 5 minutes later with 70 per cent alcohol, which was allowed to evaporate. The pulp canal was entered and the contents obtained by use of small sterile burs. The end of the bur was cut off with sterile wire-cutting pliers and dropped directly into sterile medium, which was then incubated at 37°C. No growth occurred in the medium containing normal pulp contents. When the bur was contaminated with bacteria before entering the canal, growth occurred in the medium, demonstrating that aseptic conditions were achieved and that the bacteriocidal effect of the phenol was neutralized by the alcohol.

239. Demonstration of Nerve Tissue in Periapical Inflammatory Lesions. —*James W. Bynum and Donald E. Flieder, Saint Louis University.* The purpose of this investigation was to determine the presence of nerve tissue within chronically inflamed periapical lesions. All the specimens were obtained during endodontic treatment of the involved teeth. A local anesthetic (procaine or xylocaine) was administered, and the specimens were removed surgically from around the apex of the tooth. The tissues were fixed in 10 per cent formalin for a period of 1 week or longer. The specimens were imbedded in paraffin and sectioned at 4 and 15 μ. The 4-μ sections were stained with hematoxylin and eosin and evaluated with regard to various cellular components. The 15-μ sections were stained with either Holmes's or Bodian's Protargol method. Holmes's method was employed for 98 tissue specimens. It was shown that 51 of 98 cases (51 per cent) contained argyrophilic structures having the histologic appearance and position of nerve fibers. An additional 31 cases were stained by Bodian's Protargol method. The results were more uniform and consistent with this method. It was shown that 25 out of 31 specimens (80 per cent) contained nerve tissue.

240. Biologic Effects of High-Speed Dental Cutting Instruments.—*L. S. Hansen and H. H. Scofield, U.S. Naval Dental School, National Naval Medical Center, Bethesda, and J. J. Kennedy, National Institute of Dental Research, National Institutes of Health, Bethesda.* With the advent of new dental cutting instruments there has been a resurgence of interest in the dental pulp and the effect of these newer cutting methods upon it. This experiment investigates the effect of (1) a conventional rotary instrument, (2) a conventional instrument equipped with a high-speed motor and a large pulley, (3) a water turbine, (4) two air turbines, and (5) a high-speed belt-transmission handpiece. Using 11 monkeys, Class V cavities were prepared in 244 teeth, and 61 teeth were left uncut as controls. All cavities were prepared with the same No. 35 inverted-cone tungsten carbide bur utilizing a water-spray coolant. The cavities were filled with zinc oxide and eugenol. One-third of the teeth were removed after 2 days, one-third after 14 days, and the remainder after 28 days. After fixation, the teeth were decalcified, sectioned serially, and stained with hematoxylin and eosin. Microscopic examination revealed that the significant changes consisted of displacement of odontoblastic nuclei into the dentinal tubules, loss of the odontoblastic layer, cellular infiltration of the cell-free zone, and in some teeth secondary dentin when time permitted its formation. Of the six different methods of cavity preparation, one of those using an air turbine produced a significantly greater response than the other five methods, although in all cases the reactions were mild.

241. Temperature Response to Increased Rotational Speeds.—*Alfred Schuchard and Charles Watkins, University of California School of Dentistry, San Francisco.* A review of current literature reveals uncertain conclusions relative to temperature changes in the pulp chamber with increased rotational speeds. Variations in temperature can be attributed to different investigative technics. This clinical study was instigated to determine what actually occurred in the mouth under practical conditions. The study was carried out with vital teeth which were subsequently extracted for orthodontic or prosthetic reasons. All cutting procedures were accomplished with the rubber dam in place. The thermocouple was wedged in the pulp chamber at the gingival aspect of the tooth. One hundred and forty-seven separate cutting procedures were carried out, dividing the cuts into groups: Class I, Class II, Class V, crown preparations, and cuts through amalgam and gold. Cutting was done with air alone as a coolant and air-water spray. For comparison, the first series of cuts was made at conventional speeds (1,800–10,000) with and without air coolant. Average temperature rise on all cuts was 14° F. with no coolant. Using air as a coolant, this rise was reduced to 1° F. With a belt-driven handpiece (160,000 rpm), an average rise of 5.6° F. was recorded with air alone as a coolant. Using an air turbine (290,000 rpm), we found an average drop in temperature of 5.3° F. with air alone, and a drop of 8.6° with air-water spray. We concluded from this study that increased rotational speeds were not traumatic from the standpoint of temperature rise; in fact, from this aspect it was less traumatic than conventional speed.

242. Physiologic Responses to High-Speed Handpiece Sounds.—*G. W. Rapp, Loyola University School of Dentistry, Chicago.* Modern high-speed dental handpieces produce high-pitched noises. The frequency of such noise ranges from 900 to 36,600 c.p.s. Handpiece noise increased spontaneous activity in rats from 14 to 160 motions per hour. Human hand reflex time to sight was increased by 38 per cent during 20 minutes' exposure to handpiece noise, and skilled dentists made up to 10 times as many errors when asked to trace a test pattern. Ten subjects showed an average rise of 18 mm. Hg systolic blood pressure while exposed to handpiece noise. Similar observations made while the subjects and animals were exposed to "pure" sounds of 5, 10, 12, and 15 kilocycles per second showed similar, but lower-magnitude, responses. The handpiece noise was considerably more irritating than were the individual component sounds.

243. The Effect of Noise-producing Dental Instruments on the Auditory Mechanism.—*A. K. Brenman, H. S. Brenman, S. Erulkar, and J. L. Ackerman, Department of Periodontology, Graduate School of Medicine, University of Pennsylvania, and Department of Otolaryngology, Temple University Medical Center.* Audioband spectrum analyses were performed on an ultrasonic prophylactic instrument and an air turbine. An electrode placed upon the round window of the middle ear of specially prepared anesthetized cats enabled direct recordings of cochlear microphonics and neural responses. The prepared animals were exposed to a time-controlled amount of air-borne noise generated by these instruments. Instruments were placed 14 inches from the ear of the animals. Pre- and postexposure auditory responses and neural responses were obtained. Responses were again recorded at selected times after the exposure. Audiograms were obtained from human volunteer subjects, who were then exposed to a controlled amount of air-borne noise. Postexposure audiograms were then taken. Tooth contact exposures were not made in either the animal or the human group. Animal experiments indicated an alteration in the cochlear microphonics and in the neural responses of animals exposed to these instruments. The alternative effect was more pronounced with the ultrasonic prophylaxis instrument than with the air turbine. Human volunteers exhibited audiograms with a dip in the 4,000–6,000-cycle frequency range after an exposure to the ultrasonic prophylaxis instrument. This effect appeared to be reversible within 3 days.

XXI. ENAMEL SOLUBILITY AND DENTAL PLAQUES. MARCH 20, AFTERNOON, SECTION B

244. EFFECT OF ORGANIC MATRIX ON DISSOLUTION RATES OF ENAMEL, DENTIN, AND BONE.—*Apostolos X. Apostolopoulos and Michael G. Buonocore, Eastman Dental Dispensary, University of Rochester, Rochester, New York.* To assess the role of the organic matrix on the dissolution rates of powdered enamel, dentin, and cortical bone, "intact" and "anorganized" samples of the above tissues were subjected to acid decalcification by 0.1 M acetic acid/sodium hydroxide buffers at varying pH levels and solid-solution ratios. The dissolution rates were measured as calcium and phosphorus found in the filtrates. The results suggested that the organic matrix significantly reduced the rate of dissolution of dentin and bone but not, considerably, that of the enamel. The magnitude of the effect was in the order of enamel < dentin < bone. It appeared that the solubility-retarding action increased with decreasing pH. This dissolution-retarding effect may have been due to a progressive swelling and increase in the positive charge of the organic matrix as the pH decreased. Since the dissolution process may be considered to involve a two-way diffusion through the organic matrix (acid inward and dissolution products outward), such changes in the organic matrix are likely to affect dissolution rates.

245. THE EFFECT OF SURFACE ALTERATION ON THE PERMEABILITY OF ENAMEL TO A LACTATE BUFFER.—*K. K. Kapur, Eugene Fischer, and R. S. Manly, Research Department, Tufts University School of Dental Medicine, Boston.* During studies on decalcification of enamel sections through the outer enamel surface, it was observed that slight alteration of the surface appeared to affect the rate of penetration significantly. These investigations were planned to evaluate the influence of mechanical alteration of the surface due to scratching, polishing, and grinding and the effect of topical application of sodium fluoride solution in the presence and absence of saliva. Sixty-one sections were employed, imbedded between glass slides with only the outer ends exposed to lactate buffer, 1 M, pH 5. Kodachrome transparencies were taken at thirty-two times magnification, with the section between crossed polaroids. Degree of penetration was measured by projection of the transparencies at a total magnification of 250 times. Color changes disclosed the migration of the decalcification front to the nearest micron. Roughening of the enamel surface produced a considerable increase in the rate of penetration of buffer; polishing of the roughened surface restored part of the original resistance to decalcification. Sections treated with sodium fluoride showed greater resistance to penetration up to 96 hours, but the total penetration was practically the same after 264 hours. Sections treated with water showed a slightly greater penetration than did the control sections.

246. EFFECT OF AMINO ACIDS ON SOLUBILITY OF ENAMEL AND DENTIN.—*Michael G. Buonocore, Eastman Dental Dispensary, Rochester, New York.* A series of 32 amino acids and nitrogen compounds was tested for their effect on the solubility of enamel and dentin powders in pH 4 acetate buffers. Solubilities of enamel and dentin, expressed as calcium and phosphorus in the filtrates, were determined by continuous shaking for 30 minutes in 0.1 M acetate buffer, to which had been added the acid to be tested at a concentration of 0.01 M. The results were compared with the amount of dissolution in plain 0.1 M acetate buffer and expressed as percentage solubility change. Of 32 compounds tested, a significant reduction (over 10 per cent) in enamel solubility was shown by glutamic acid (11 per cent); glutathione (15 per cent); DL-lysine HCl (18 per cent); aspartic acid (30 per cent); DL-aminophenyl acetic acid (12 per cent); and amino hippuric acid (15 per cent). Increasing the concentration of DL-lysine to 0.02 M almost doubled the 0.01 M solubility reduction (34 per cent). A 0.02 M concentration of aspartic acid produced a (37 per cent) solubility reduction. With dentin, only amino hippuric acid significantly reduced solubility (10 per cent). A similar difference in behavior between enamel and dentin was also observed and reported previously by the author for a series of 15 nitrogen-free organic acids.

The greater sensitivity of enamel to the composition of an acidic environment indicates that the nature of the acids present in the buffer systems of plaques may be of considerable importance in the rate of enamel decalcification in caries but not of dentin.

247. Dissolution of Enamel by Lactic Acid and Succinic Acid Buffers.—*Winfrey Wynn, John Haldi, and Mary L. Law, Emory University Dental School, Atlanta.* Intact human enamel surfaces were exposed in vitro for 2-week periods to 0.1 per cent lactic acid and succinic acid buffers adjusted to pH 4, 5, and 6 at 37° C. The buffer solutions were changed each day, pooled, and evaporated to dryness for analysis for Ca, P, and Mg. The chalky enamel surfaces, after soaking, were scraped free of the partially decalcified enamel, and this material was collected, dried, and ashed for analysis for Ca, P, Mg, and CO_2. The succinic acid buffers decalcified the enamel twice as rapidly at pH 4 and four times as rapidly at pH 5 and 6 as did the lactic acid buffers. Ca and P were removed by both buffers from the enamel at a Ca/P ratio of 2.0–2.1, which is the ratio we found in intact human enamel. The amount of CO_2 removed was 1.5 per cent by both the lactic acid and the succinic acid buffers. There was a greater proportional loss of Mg and CO_2 than there was of Ca and P. This would seem to indicate that the Mg and CO_2 were preferentially leached out of the enamel by both the lactic acid and the succinic acid buffers. The loss on ashing the chalky enamels obtained by partial decalcification by both buffers at pH 6 was greater than at pH 4 or 5, indicating that the content of organic matter was greater in chalky enamel obtained at the higher pH.

248. Pyrophosphate, a Factor in Tooth Erosion.—*John Madonia and G. W. Rapp, Loyola University School of Dentistry, Chicago.* Pyrophosphate is a "natural" chelating agent which holds calcium in solution in a non-ionized form. The shift in total calcium capable of remaining in phosphate-containing solutions is thus increased. Pyrophosphate, in the presence of tooth substances, removes calcium from the solid phase over a wide pH range, including neutrality. Fermentation of glucose by whole saliva, salivary debris spun down from whole saliva, and a mixed culture of oral microorganisms caused the accumulation of 7–15 mg., 16–190 mg., and 260–330 mg. of pyrophosphate, respectively, under conditions of our test. The pyrophosphate thus accumulated, when allowed to act on powdered tooth enamel, solubilized 10–35 mg. of calcium per 100-ml. reaction mixture.

249. Distannous EDTA and Its Effect on Dental Enamel.—*H. G. Langer, Dow Chemical Company, Eastern Research Laboratory, Framingham, Massachusetts.* Previous reports on caries inhibition have shown the importance of the stannous metal ion in SnF_2 as well as the fluoride ion and the effectiveness of some metal ion chelates, including Zn, Ni, and Mn versenate. A new chemical, the distannous chelate of EDTA, has been prepared, which combines the effect of stannous ions with the advantages of the chelate—low toxicity and rate of hydrolysis as compared with other tin (II) salts. The compound has a surprisingly low solubility in water; yet, upon heating an aqueous slurry of dental enamel and the chelate for Ca/Sn 1/1, quantitative formation of a basic stannous phosphate takes place, corresponding with stannous fluoride treatments. Rat feeding tests by L. C. Hendershot, R. Mansell, and J. Forsaith (Biochem. Res. Lab., Dow Chemical Company) show a definite dose-response relationship for different amounts of stannous EDTA added to the cariogenic diet and a significant caries inhibition in female rats, starting with a level of 200 ppm Sn. A highly significant reduction in enamel solubility was obtained through treatment with $Sn(II)_2$ EDTA by R. S. Manly (Westwood Research Laboratory).

250. Rehardening of Artificially Softened Enamel.—*Theodore Koulourides and Ward Pigman, University of Alabama Medical Center, Birmingham.* Remineralization of human enamel softened by treatment with acetate buffer (0.001 M, pH 5.5) at 37° was accomplished by treatment with calcium phosphate solutions. The

softening and remineralization were followed by measurements of hardness.* The rehardening was accomplished by exposure of softened enamel surfaces to solutions of secondary and tertiary calcium phosphates having calcium concentrations of 1–3 mM per liter and pH's of 6.8–7.2. For the preparation of these solutions, the salts were first dissolved in hydrochloric acid, and the pH was raised by addition of potassium hydroxide to pH values which were found optimum for rehardening for each calcium concentration. Most of the solutions produced precipitates either immediately after their preparation or within 24 hours. Complete recovery of hardness was obtained with both secondary and tertiary calcium phosphates. Tricalcium phosphate gave a faster recovery than did dicalcium phosphate, and the rate was further accelerated by addition of fluoride (in molar ratios Ca/F of 5/1). In the presence of potassium fluoride, rehardening took place within 4 hours at 37°. In a typical instance, enamel with an initial hardness of 300 KHN was softened to 200 KHN and rehardened to 300 KHN. The recovery of hardness was not complete when enamel was softened below 170 KHN. Possibly this represents a critical limit which may be an index of an initial carious lesion. Original and rehardened enamel had similar rates of softening when exposed to acid buffers.

251. Retention of Substrates by Layers of Salivary Sediment.—*R. S. Manly, Department of Dental Research, Tufts University School of Dental Medicine, Boston.* A study was performed on retention of glycolytic activity that is induced by treatment of thin films of sediment and plaque with sucrose and glucose. The experiments involved high and low molarity of sugars and contact times ranging from 3 to 30 minutes. Sediments were placed in contact with glass electrodes and allowed to come to steady state in bicarbonate buffer containing supernatant liquid from saliva. Glucose and sucrose solutions at concentrations equivalent to 4 M glucose were used to treat the sediment for either 3 or 30 minutes. Aliquots were treated similarly with low concentrations of sugar (equivalent to 0.2 M glucose). The average finding from ten such experiments showed that 2 M sucrose produced significantly longer pH drop than did the glucose at equivalent concentration. A repetition of this experiment with individual sediments showed a slightly greater, but statistically significant, effect for sucrose in maintaining the pH drop both with high (16 subjects) and low (8 subjects) concentrations of sugar.

252. Effect of Cell Concentration on Dental-Plaque Metabolism.—*I. Kleinberg, Department of Biochemistry, University of Manitoba, Winnipeg, Canada.* It was found earlier (I. Kleinberg, *J. D. Res.*, **37**:756, 1958) that the effect of different concentrations of glucose on dental-plaque pH in vivo was similar, at least qualitatively, to results found in vitro with incubated saliva. In both these systems, at lower glucose concentrations the rate of acid production was directly related to the glucose concentration, with a maximum being reached at a concentration of approximately 5 per cent. However, at higher glucose concentrations, both in plaque and in incubated saliva, inhibition of acid production was observed, but this inhibition was appreciably less with plaque, in which inhibition was not evident until very high concentrations were reached. To determine whether their difference in bacterial-cell concentration could account for this difference in these two systems, saliva-glucose mixtures containing different cell and glucose concentrations were incubated at 37° C. for given time intervals, and the pH values produced were compared. It was found that, as the cell concentration in the saliva-glucose mixtures was increased, the shape of the pH curves approached that found with plaque in vivo, so that, with relatively high cell concentrations in the mixtures, only a slight inhibition of acid production occurred at high glucose concentrations, just as was found with dental plaque in vivo. In addition, the observations seemed to indicate that higher glucose concentrations had a greater inhibiting effect on bacterial growth than on bacterial acid production.

*A Tukon Microhardness Tester was used.

253. Retention Properties of Foods Investigated by a Centrifugal Technic.—*Donald J. Beck and Basil G. Bibby, Eastman Dental Dispensary, Rochester, New York.* Using a centrifugal technic described in a previous paper (*J. D. Res.*, **38**: 704, 1959), some of the factors which affect the retention of foods to the teeth were investigated. The speed and period of centrifugation were shown to have a profound effect on the retention values obtained, significant reproducible results being achieved only when a relatively high speed and long period of centrifugation (900 rpm for 15 minutes) were employed. The amount of saliva in the test sample was shown to affect the retention index, but this effect varied from food to food. Twelve representative foods, each tested at varying consistencies (i.e., mixed with varying amounts of saliva), were investigated by the centrifugal retention technic. This study has served to emphasize the complexity and variability of factors affecting the retention of foods to the teeth.

254. A Method of Inducing Migration of Leukocytes Associated with Plaque.—*J. D. Wildman, Syracuse University Research Corporation, Syracuse, New York.* Certain cellular and colloidal components of dental plaque appear not to have been studied extensively. These consist of epithelial-like cells of varying sizes, leukocyte-like cells, and non-cellular components consisting of webs, strands, rounded amorphous gels, and the gel material which tends to hold the organisms. When a small piece of soft plaque is pressed out gently in saliva, the leukocytes will often be found in varying numbers on the outer margin of the amorphous material. They may also be mixed with the bacterial gel. Leukocytes, when examined in saliva, are usually spherical, not amoeboid, and rarely migratory. It was found that when plaque was mounted in a modified Ringer's solution containing sodium hexametaphosphate, the leukocytes usually showed vigorous amoeboid and phagocytic activity and that some of them migrated across the field. Examination of specimens of plaque from forty individuals showed that, in general, leukocytes in saliva are spherical but show migratory activity in the modified Ringer's solution. Time-lapse motion pictures revealed random motion of cells and the migration of some of the cells into the amorphous bacterial masses. This observation showed that the leukocytes were alive as they entered the bacterial mass and therefore played some part in the metabolic activity of plaque. Although leukocytes in saliva do not usually show migratory motion, it is possible that *in situ* their activity may differ from their activity in an isolated drop of saliva.

XXII. PERIODONTOLOGY. MARCH 20, AFTERNOON, SECTION C

255. Histopathology of Periodontal Disease in Hibernating and Non-hibernating Ground Squirrels.—*Wen-Shui Shih and Alton K. Fisher, College of Dentistry, State University of Iowa, Iowa City.* This study was based on examinations of jaws of 8 wild animals of unknown age, 10 non-hibernating animals 3 years old as controls, and 10 animals 3 years old that hibernated 4 months in each year. Hibernating animals were sacrificed 6 hours after emerging from hibernation. Extraneous periodontal deposits included considerable calcareous material in wild squirrels, whereas those of control and hibernating animals contained mostly food debris and hair, the latter predominating. While some slight periodontitis and occasional superficial ulceration were observed in wild animals, periodontitis of various degrees was four to five times as frequent in control and hibernating animals. Periodontal pockets were not observed in the wild animals but were frequently noted in the caged animals. Wild squirrels showed no missing teeth or present root fragments, whereas slightly less than half of the lower teeth of both groups of caged squirrels were represented only by retained root fragments. It was not readily apparent why these teeth were broken or exactly how much they contributed to periodontal disease. Boredom and cage-chewing may have been contributing factors. Boredom and licking of fur may also have caused the hair impactions. Bone marrow was predominantly hematopoietic in wild squirrels, slightly more fatty in the controls, and still more fatty in hiber-

nators. A mosaic character of interradicular bone was moderately obvious in caged animals, apparently related to inflammatory changes. This was also true of free mucopolysaccharides. Osteogenetic activity seemed generally similar in all groups, but the thickness of osteoid layers was definitely greater in caged animals, suggesting nutritional disturbances. It was concluded that caging rather than hibernation has a greater adverse effect on the periodontium.

256. Histologic Studies of Periodontal Disease in the Rice Rat.—*Om P. Gupta, University of Illinois College of Dentistry, Chicago, and James H. Shaw, Harvard School of Dental Medicine, Boston.* Gross observations of periodontal lesions in the rice rat have revealed a high susceptibility of this species to periodontal disease. Detailed histologic studies were conducted to determine the stages of progress of periodontal disease in this animal. Seventy-five rice rats were bred under controlled conditions and were maintained on a purified ration for a period ranging from 8 to 30 weeks. A few animals were killed each week after the age of 8 weeks. The jaws were X-rayed, examined grossly, serially sectioned, and stained with hematoxylin and eosin, Mallory, Van Gieson, and silver stains. Susceptible and resistant strains were revealed by histologic observation. Sixty-two per cent of the animals were susceptible. Animals in a susceptible litter showed extensive lesions as early as 11 weeks, while resistant litters did not show any appreciable pathologic disturbance even at 27 weeks. Periodontal lesions were characterized by inflammatory cells and edema in the epithelial and subepithelial layers, dilatation and engorgement of blood vessels, connective tissue edema, periodontal membrane thickening, and crestal resorption. About 20 per cent of the animals showed migration, diastematas, degeneration and necrosis of gingival and periodontal fibers, and extensive bone resorption. A few resistant animals showed mildly pathologic alveolar bone. The results suggest that the susceptibility to periodontal disease in the rice rat is associated with genetic factors. The usefulness of the rice rat in periodontal research is thus further confirmed.

257. Effects of Hypervitaminosis D on the Periodontium of the Hamster.—*W. E. Rogers, H. Fahmy, and David F. Mitchell, Indiana University School of Dentistry, and Indiana University Medical Center, Indianapolis.* Sixty young adult hamsters were divided equally by sex and with absolute littermate distribution into three groups: experimental, pair-fed, and ad libitum–fed control. Each experimental animal received an intraperitoneal injection of 5,000 I.U. of calciferol in sesame oil twice weekly for 16 injections (80,000 I.U., 8 weeks). Water and laboratory chow were available ad libitum to the experimentals and the ad libitum controls. The daily food intake was measured for each animal in these two groups, and the amount of diet consumed by each experimental hamster each day was given to its pair-fed control the following day. The animals were weighed every other day. The experimental animals exhibited anorexia and weight loss greater than those pair-fed, while the ad libitum controls gained weight. The plasma calcium of the experimental animals was markedly higher than that in both control groups, and the ash wt/dry wt per cent of the femurs of the experimentals was less than that of both control groups. No significant changes were detected in the salivary glands. Kidneys showed vasodilatation and pathologic calcification involving the tubules, interstitial tissue, and glomeruli in a decremental order. The degree of kidney affection paralleled the severity of the hypervitaminosis. Femurs revealed resorption and thinning of the cortexes. Fatty marrow changed toward cellular marrow. The molar periodontium showed unusual osteoid, calcification of Sharpey's fibers, and resorption of interdental, interradicular, and central mandibular bone, which paralleled the severity of the hypervitaminosis. The incisor sections also exhibited most of these changes.

258. Regeneration of Gingival Tissue in Non-human Primates.—*Frederic M. Chacker and D. Walter Cohen, Department of Periodontology, Graduate School of Medicine, University of Pennsylvania, Philadelphia.* This study, using African green and mangabey monkeys, was concerned with (1) topographic changes in

regenerating gingival tissues, (2) apparent changes in the width of the attached gingival band, and (3) histologic evidence of changes in the configuration of the tissue due to alterations in environment. The three molars of the mandibular right quadrants were marked by notching with a diamond stone. The distances between the notches and the gingival margin and between the notches and the mucogingival junction were measured and noted. The animals were subjected to a commonly employed form of mucogingival surgery. The attached band of gingiva on the buccal surface of the four posterior teeth in the mandibular right quadrant was removed by gingivectomy. (The tissue was preserved in 10 per cent formalin and sectioned after staining.) After making two vertical-relaxing incisions, the soft tissue below the area of gingivectomy was carefully dissected from the underlying bone. The resultant flap of tissue was replaced in a position apical to the original location, and the distance from the cut edge of tissue to the notch on the teeth above was measured and noted. The exposed bone was severed and a surgical pack placed over the area; wire ligation was used to promote retention. The pack was replaced weekly for 3 weeks. The animals were sacrificed by perfusion of 10 per cent formalin (following 0.9 per cent saline) into the two common carotid arteries. Termination of the experiment was at 10 days, 3 weeks, 6 weeks, 3 months, and 6 months postoperatively. Measurements of the previously noted distances were made regularly during the experiment and immediately preceding the perfusions. Photographs of the areas were made with the measuring probe in place. An ultrafiltrate of India ink was used in a group of animals, in order to perfuse the vascular supply of the regenerating tissue. Formalin-perfused groups were stained with hematoxylin and eosin or with ercinol (elastic tissue) stain. The perfused specimens were cleared and sectioned without decalcification, to observe the vascular patterns. The results of these studies indicated that the mucogingival was placed more apically in the 3-month experimental animal. The approximate average of increased attached gingiva resulting from this procedure was 2 mm. Complete epithelialization was not observed in less than 6 weeks.

259. Studies on Periodontal Disease in the Mouse. III. The Germ-free Mouse and its Conventional Control.—*Paul N. Baer, National Institute of Dental Research, and Walter L. Newton, Laboratory of Germfree Animal Research, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda.* We have shown previously that many strains of mice are susceptible to periodontal disease. The purpose of this investigation was to study the extent of the contribution made by the oral flora in the etiology of this disease. The material used consisted of the jaws and teeth of 38 germ-free and 20 conventional white Swiss mice, ranging in age from 1 to 6 months. They were all fed the same nutritionally adequate diet. After sacrificing the animals, the upper and lower molar teeth and jaws were studied grossly and microscopically as previously described. Results to date are as follows: (*a*) Gross observations: the defleshed specimens of all conventional and germ-free mice showed loss of alveolar bone starting at approximately 4 months of age and increasing in severity with increase in age. (*b*) Microscopic observations: in mesiodistal sections in both conventional and germ-free animals, 1 through 6 months of age, the epithelial attachment formed a butt joint with the most coronal portion of the cementum at the cementoenamel junction. The alveolar bone was normal in appearance. In buccal-lingual sections of both germ-free and conventional animals, however, there was a marked difference between the 1- and 6-month-old animals. The younger animals had epithelial attachments located at the cementoenamel junction, while the older ones, particularly on the lingual surfaces, exhibited considerable downgrowth of the epithelial attachment, bone loss, and pocket formation. There were no apparent differences in the periodontal conditions of the animals, however, whether they were raised in a conventional manner or were germ-free.

260. Incidence of Periodontal Disease among Persons with Neuropsychiatric Disorders.—*Charles M. Belting, Veterans Administration West Side Hospital,*

Chicago, and Om P. Gupta, University of Illinois College of Dentistry, Chicago. Clinical impressions reported in the literature have suggested a higher incidence of periodontal disease in persons suffering from emotional and psychiatric disturbances. In order to test this hypothesis, a periodontal survey was made of 105 patients receiving psychiatric treatment and 117 patients receiving medical care other than psychiatric treatment at the Outpatient Clinic of the Veterans Administration West Side Hospital. All patients were male veterans between the ages of twenty and sixy-five. The periodontal lesions were evaluated by the method of Russell (*J. D. Res.*, **35**:350, 1956), without X-rays, using a mouth mirror and a periodontal probe under good lighting. The vital statistics recorded included frequency of brushing, degree of calculus, bruxism and clenching habits, income, educational level, psychiatric and/or medical diagnosis, and medications received. The experimental group of psychiatric patients showed a higher incidence of periodontal disease than did the control group of non-psychiatric patients in all age groups between twenty and sixty-five. The increase was highly significant in each age group up through age fifty. In order to eliminate the variable factor of oral hygiene care as a possible cause affecting the difference in incidence among the two groups, those persons who brushed their teeth once a day and also those who brushed their teeth twice a day in each group were compared. Among those persons brushing their teeth once a day, the incidence of periodontal disease in the experimental group was significantly higher than in the control group at all ages between twenty and fifty years. Among those persons brushing their teeth twice a day, the incidence of periodontal disease also was significantly higher in the experimental than in the control group, with the exception of the years between thirty-one and forty.

261. Periodontal Disease in a Group of Mentally Subnormal Children.—*M. Michael Cohen, Richard A. Winer, and Gerald Shklar, Boston Dispensary Rotch Research Laboratory.* The periodontal condition was evaluated in 300 male and female institutionalized, mentally subnormal children aged three to eighteen. This group consisted of both Mongoloid (100 cases) and non-Mongoloid patients (200 cases). All cases were carefully studied clinically, and color photographs were taken. Of this series, 20 cases were selected at random for further evaluation. Full-mouth intraoral radiographs and gingival biopsies were obtained in each case. The medical histories were carefully reviewed. All the Mongoloid children were found to have periodontal involvement varying in degree from moderate to severe. In addition to periodontitis, a superimposed necrotizing gingivitis was noted in many cases. There was radiographic evidence of bone loss in all cases studied. This was characterized by horizontal resorption and loss of crestal lamina dura. There also appeared to be an altered trabecular pattern in many of the cases. Microscopic studies of the gingiva revealed nonspecific inflammatory disease varying in degree from moderate chronic inflammatory infiltration of the connective tissue to more severe cases in which there was a thinning and degeneration of the epithelium, as well as an intense inflammatory infiltration. Oral hygiene was poor and calculus, materia alba, abundant, but it was apparent that tissue breakdown was considerably more severe than expected in this age group despite the local factors present. Nutrition in this group was excellent and has been carefully evaluated. Periodontal disease was more prevalent and more severe in the Mongoloid group than in the non-Mongoloid, mentally subnormal patient.

262. Dimensions of the Dentogingival Junction in Humans.—*Anthony W. Gargiulo, Frank M. Wentz, and Balint Orban, Department of Periodontics, Loyola University School of Dentistry, Chicago.* An attempt was made to give an actual measurement to the component parts of the dentogingival junction. Normal human autopsy block specimens were studied. The subjects ranged in age from nineteen to fifty-six years. The following measurements were ascertained, and an ideal structural relationship for the four phases of passive exposure was established. The following measurements were made: (1) sulcus depth, (2) length of the attached epithelium,

(3) the most apical point of the attached epithelium below the cementoenamel junction, (4) distance from the bottom of the sulcus to the cementoenamel junction, (5) distance from the cementoenamel junction to the crest of the alveolar bone, and (6) distance from the deepest point of the attached epithelium to the alveolar bone. The figures ascertained were averages of all specimens in all phases and also average values for each of the four phases of passive exposure. The results were (1) sulcus depth, 0.69 mm.; (2) length of attached epithelium, 0.97 mm.; (3) attached epithelium below the C-E junction, 0.64 mm.; (4) distance from sulcus depths to the C-E junction, 0.79 mm.; (5) distance from the C-E junction to the alveolar bone, 1.78 mm.; and (6) distance from the deepest point of the attached epithelium to the alveolar bone, 1.13 mm. The value for total attachment was 2.10 mm., and total length of the dentogingival unit, or magnitude, is given by the phases as I, 3.23 mm.; II, 3.06 mm.; III, 2.41 mm.; and IV, 2.53 mm. The average age for each phase was: I, 24.5 years; II, 31.4 years; III, 32.3 years; and IV, 39.7 years.

263. Epithelial Rests in the Periodontal Ligament of Humans.—*Charles M. Reeve, Loyola University School of Dentistry, Chicago.* This investigation is based on the histologic study of the epithelial rests in the periodontal ligaments of 500 teeth from 57 human autopsy specimens ranging from one to seventy-seven years of age. All specimens demonstrated epithelial rests, the incidence decreasing with age. Three morphologic types were noted: (1) a small resting type, (2) a proliferated type, and (3) a degenerated type. It was assumed that the three morphologic types represented different stages in the life-cycle of the epithelial rests. The distribution of the rests varied with age; the location of the majority of the rests was in the cervical area in all age groups except those from eleven to twenty years. While the majority of the epithelial rests were derived from Hertwig's epithelial sheath, some may have originated from the epithelium of the gingiva or from the epithelial attachment.

264. Effects of Auditory Stress upon Healing of Experimental Periodontal Lesions.—*Donald B. Giddon and Paul Goldhaber, Harvard School of Dental Medicine, Boston.* It has been demonstrated that wound healing of experimental skin lesions may be retarded in 5–6-week-old seizure-susceptible strains of mice subjected to auditory stress. The present preliminary study represents an attempt to demonstrate a similar influence of this neurogenic stressor on healing of oral tissues produced experimentally by electrocoagulation. 120 DBA/1 mice were equally divided into a stressed and a non-stressed group. The stressed group was subjected to intense auditory stimulation of approximately 90 seconds' duration on each of 5 successive days. On the fifth day, a gingival lesion was produced between the lower incisors in both groups. Auditory stimulation was continued in the stressed group for a total of 18 days, at which time all remaining animals were sacrificed. During the healing period the oral lesions were grossly examined and scored. After sacrifice, the adrenals were weighed, and the periodontal lesions were examined histologically. Although it appeared that auditory stimulation was a stressor, as indicated by a significant increase in adrenal weights of the stressed animals as compared with the non-stressed group, no gross or microscopic differences in healing were observed. Failure to demonstrate impaired healing of oral lesions in response to auditory stress might be explained in part by variability in severity of the initial lesion, as well as inherent differences between oral tissues and skin.

265. Microrespirometer Technic for Study of Human Gingival Tissue: Preliminary Report.—*J. H. Manhold, Jr., T. E. Bolden, and S. Katz, Seton Hall College of Medicine and Dentistry, Jersey City.* Glickman, Turesky, and Hill (1949) employed the Warburg manometric technic to study human gingival metabolism. Because accurate Warburg determinations require relatively large quantities of tissue (40–50 mg. wet weight) and such quantities of human tissue usually are not readily available, Glickman, Turesky, and Manhold (1950) employed dogs to study oxygen requirements of healing gingival tissue. Exact relationships between human and dog gingiva Q_{O_2} values were not found. Further investigations did not follow. Kirk (1950)

presented a quantitative ultromicroanalysis method for measuring the metabolism of tissue cultures. This technic, giving accuracy on 10 mg. or less (wet weight) of tissue, appeared ideal for human gingival study and was used in the present investigation. Small sections of gingival tissue, removed from clinic patients of Seton Hall College of Medicine and Dentistry, were placed in Tyrode's solution and divided into two approximately equal parts. Half was fixed for microscopic study. Half was placed in microrespirometers for Q_{O_2} determinations. Thirty-six samples of microscopically determined normal and essentially normal gingival tissue yielded a mean Q_{O_2} of 1.39 ± 0.46, a value comparable to earlier results. Microrespirometer investigation of human gingival tissue appears feasible, therefore, and presents the advantage of using small tissue quantities.

266. Toothbrushing Instruction in Primary Grades.—*J. E. de Wever, Professional and Educational Services, Colgate-Palmolive Company, Jersey City, and H. M. Averill, Eastman Dental Dispensary, Rochester, New York.* Three groups, each consisting of two first grades and one second grade, were selected at random from three public schools in Webster, New York, to evaluate a specific outline of classroom instruction in toothbrushing. One dental hygienist gave three 20-minute sessions of instruction a week apart to each class in groups A and B. The children of group B also brushed their teeth after school lunch under the supervision of the classroom teachers. Neither instruction nor practice was given group C. Applying the brushing method advocated by the American Dental Association, the instructor demonstrated on cheek and lips the actual hand positions and wrist movements used in each upper and lower area of the mouth. Pupil exercises followed each step of the demonstration. The first session covered the labiobuccal area, the second added the lingual, and the third included all areas. Two observers recorded the brushing method and which of the 6 buccal, 6 lingual, and 4 occlusal areas the child attempted to clean. All children were individually observed at the beginning of the study, after the last session of instruction, and 3 months later. The results were: (1) 75 per cent of the pupils in group A and 85 per cent in group B brushed in the instructed method; (2) both groups increased by 85 per cent the number of lingual areas brushed; (3) 3 months later the same results proved an excellent memory retention; and (4) first- and second-graders learned a proper method of toothbrushing in a total of 1 hour of instruction.

XXIII. SYMPOSIUM I. ABSTRACTS NOT AVAILABLE

XXIV. SYMPOSIUM II. CLINICAL RESEARCH. MARCH 18, EVENING

S13. A Basis for Establishing Rapport and Successful Dental Management of Brain-injured Children.—*Jerry J. Adelson, Jewish Memorial Hospital, New York.* This paper is concerned with and limited to those areas in special-education methods for brain-injured individuals as applied to dental management. These modified methods may be successfully practiced by the dental practitioner without extensive training. The range of application is defined as follows: all brain-injured children who demonstrate the behavioral characteristics of "organicity" but who may or may not be mentally retarded and/or physically handicapped. Clinical observations of these children have yielded evidence of specific disturbances in perception, thinking, and emotional behavior, either separately or in combination. These unique behavioral characteristics present obstacles to successful direction through communication—a basic prerequisite to the establishment of rapport and dental management. This behavioral syndrome pattern consistent with these brain-injured individuals requires identification, recognition, and understanding to support the gainful dental experience. A clinical investigation was undertaken to test the validity of specific special-education technics and procedures transposed to a dental milieu. Although the clinical sampling has been limited to date, the evidence points to the gainful and rewarding applications of the methods discussed.

S14. The Etiology of Recurrent Aphthous Stomatitis: The Effects of a Non-allergenic Regime in Hospitalized Patients.—*Irwin I. Ship, National Institute of Dental Research, National Institutes of Health, Bethesda.* The appearance of recurrent aphthous ulcerations on the oral mucous membranes in susceptible individuals following the ingestion of certain foods has been frequently noted. As a result, allergy to food substances has been suggested as being the primary etiologic factor responsible for recurrent canker sores in the mouth. Seven patients with severe recurrent aphthous ulcerations were admitted to the Clinical Center, National Institutes of Health, for study. Various biochemical, hematologic, immunologic, microbiologic, and pathologic studies were performed. All aromatic oils in tooth and skin preparations were eliminated during the course of study, and a highly purified, relatively non-allergenic dietary regime was maintained for periods ranging from 2 to 4 weeks. The diet consisted of distilled water, sucrose, purified corn starch and corn oil, purified vitamin-free casein, salt, and artificial flavoring materials. Purified vitamin preparations were included in the diets of three of the seven patients. The location and duration of all aphthous ulcerations were recorded on special diary forms for periods ranging from 1 to 8 months prior to hospital admission, during the hospital stay, and for similar periods following discharge. Oral ulcerations failed to develop in six patients during the first week, although their patterns suggested continuous ulcerations prior to admission. All six developed no ulcerations during the study period.

S15. A Different Approach to the Closure of Oro-antral Fistulas, Using a Gold Plate.—*Fred Rothenberg.* After antral infection was eliminated, an open oro-antral fistula was closed for each of ten patients, using a 24-carat, 36-gauge gold plate extending 2 or 3 mm. beyond the margin of the bony defect. The edges of the plates were placed between the periosteum and the tissue and sutured into place, but with no attempt to close the external opening, so that the gold was visible in most cases. The plate was pulled out without anesthesia after 2–3 weeks. On removal of the plate, the bony defect was found closed by a dense, hard, red tissue. This tissue later turned pink, and the surface tissue defects disappeared, the wound healing completely. There was little postoperative discomfort or swelling and little bleeding. On section of the tissue closure formed under the gold no foreign-body reaction was found. Additional cases are being treated in this way.

S16. A Five-Year Study on the Use of Phenoxymethyl Penicillin (Penicillin V).—*Harold M. Syrop, F. B. Wiesbusch, and Sim Chappelle, Medical College of Virginia, Richmond.* This study was undertaken, for the most part, to evaluate the effectiveness of an orally administered penicillin (Penicillin V) for prophylactic use in rheumatic fever, valvular and congenital heart lesions, renal disorders, and poorly controlled diabetics. These patients were to undergo routine exodontics and minor oral surgery, periodontal therapy, and endodontic procedures. Acute inflammatory periapical involvement with elevated temperature, spetic facial cellulitis, and acute pericoronitis with marked lymphadenopathy and fever were also therapeutically resolved by Penicillin V. Phenoxymethyl penicillin was likewise employed as an adjunct for severe, acute necrotizing ulcerative gingivitis with high fever and malaise and also in some extenuating endodontic cases. Patients with past histories of hives, urticaria, hay fever, asthma, bronchitis, serum sickness, food and drug sensitivity, previous reaction to penicillin, and so-called "Id" reactions were given erythromycin instead of penicillin. Since 1955 and up to the present, no cases of subacute bacterial endocarditis have been reported among our patients, and, except for one incident where the patient had received two types of penicillin consecutively with subsequent urticaria, no accidents of anaphylaxis, serum sickness, and untoward skin rashes as a direct result of Penicillin V therapy were noted. It appears that the ease and convenience of administration, absence of pain, procurement of adequate penicillinemia, elimination of the possibility of inoculation, hepatitis, and maintenance of adequate serum levels at home by the patient pre-empt the intramuscular use of Penicillin V in favor of oral penicillin for ambulatory dental patients without gastrointestinal disorders.

S17. The Response of the Gingival Sulcus to Instrumentation: A Histologic Investigation.—*Bernard S. Moskow, Boston University School of Medicine.* Among the available therapeutic measures the periodontist has to choose from are scaling and curettage. Although extensively used, there remains, both in practice and in the literature, much confusion as to the rationale, technics, and methods and indications for the performance of these procedures. More important, the abundance of views expressed on the nature of the response of the gingival sulcus to these measures has resulted in a rather perplexing approach for the practitioner. In nearly 500 areas of the mouth where gingivectomy was to be performed, the gingival sulcus was instrumented, using either the scaling or the curettage procedure. The areas were operated either immediately prior to, or from periods of 1 day to 4 months before, surgical intervention. In most instances the procedure was performed on the day of the gingivectomy. In this way, both the effect of the instrumentation on the sulcular tissue and healing following the performance of these measures could be evaluated. An attempt was made to correlate the histologic picture with the clinical type of gingivae that would respond favorably to instrumentation. Results indicate that subgingival curettage as a distinct and separate procedure will in most cases remove all the sulcular epithelium and the epithelial attachment. The latter appears to be removed in routine operation of the sulcus. Scaling, however, will not accomplish complete epithelial debridement of the gingival sulcus but rather a tearing or splitting of the epithelial rete pegs with partial removal of the sulcular epithelium. Healing in the form of continuity of the epithelial lining was observed within 1 week following instrumentation.

S18. Studies on a Hydrogenated Fat-Zinc Bacitracin Periodontal Dressing.—*Charles F. Sumner III, Paul N. Baer, and John Scigliano, Walter Reed Hospital and National Institutes of Health, Washington, D.C.* Periodontal surgery designed to reposition the mucogingival tissue often results in exposure of alveolar bone. It is not unusual that such surgically exposed areas require 1, 2, or more weeks for granulation tissue completely to cover exposed bony surfaces. Since this delay in healing might be due, in part at least, to the eugenol content of the dressing, the purpose of this investigation was to develop and clinically test a periodontal dressing which did not contain eugenol. Patients with a diagnosis of gingivitis or periodontitis who required periodontal surgery were used as the subjects. Controls were run as follows: after the surgery was completed, a control dressing containing eugenol was placed over one half of the area, while the experimental dressing containing hydrogenated fat was placed over the remaining half. At the end of 1 week both dressings were removed. The results were as follows: (1) No allergic reactions or *Candida* infections were seen in either group. These observations are based on a total of 735 cases in which zinc bacitracin was incorporated in the powder. (2) The experimental dressing was easier to handle and more comfortable to the patient than the control. (3) In one-fourth of the cases involving extensive exposure of alveolar bone, the healing seemed to be at a more advanced stage at the end of the first week. (4) The control pack developed sharp, annoying edges in 5 per cent of the cases, while the experimental dressing did not develop these hard, sharp edges at any time.

S19. Infrared Electronics in Oral Diagnosis.—*Joel Friedman, Jewish Chronic Disease Hospital, Brooklyn, New York.* Research and development in military electronics have brought bountiful by-products in industrial and medical applications. A number of potential areas in dentistry are suitable for investigation into the uses of electronics in evaluating the state of oral tissues in health and disease. Infrared radiation, more penetrating than visible light, has aided diagnosis in peripheral circulatory disease, dermatologic lesions, and particularly ophthalmologic problems. More recently, infrared instrumentation has been developed for reading body-surface temperature to within a fraction of a degree from a Polaroid print. By means of this record of superficial heat distribution, differential diagnosis of breast cancer is frequently facilitated. Infrared photographic studies of venous patterns in the oral sub-

mucosa have confirmed the potential of this approach to the study of oral pathosis in patients with systemic circulatory disease. For basic anatomic studies of normal and abnormal mucosal venous patterns, this approach, utilizing direct infrared electronic viewing, holds promise. Inflammatory gingival disease, venous stasis, mucosal changes in puberty and pregnancy, and studies of the effects of drugs on oral tissues are other avenues for research. Advances in new instrumentation and new applications for direct infrared electronic examination, temperature studies, and spectrophotometric analyses of tissue chemistry require that dentistry begin to avail itself of this tool and systematically examine its possibilities in the field of mucosal and hard-tissue pathology, as yet unexplored by the use of infrared electronics.

S20. Electromyographic and Clinical Investigation of Dysfunctional Pain in the Temporomandibular Joint and Adjacent Muscles.—*Sigurd P. Ramfjord, University of Michigan School of Dentistry, Ann Arbor.* Over a period of 3 years, thirty-two patients with temporomandibular joint symptoms and muscle pain were examined clinically and electromyographically before and after occlusal adjustment. Various degrees of bruxism associated with psychic tension and occlusal interferences were observed in all the patients. Clinically, all the patients experienced relief of their pain and discomfort following complete occlusal adjustment, and electromyographically a harmonious and well-synchronized contraction pattern of the temporal and masseter muscles was recorded after the adjustment. Any type of occlusal interference was found to trigger muscle spasms and pain, but the most consistent and significant interference was a discrepancy between centric relation (posterior terminal hinge position) and centric occlusion (maximum occlusal contact), with the muscle disharmony displayed electromyographically mainly in swallowing. The second most significant occlusal disharmony was balancing side interference. The balancing side contact had to be lighter than the working side contacts in order to establish a harmonious masticatory pattern. In no instance was "distal displacement" or "mandibular overclosure" the cause of the temporomandibular joint and muscle disturbances. A very close correlation was observed between the clinical and electromyographic recordings of occlusal interferences and the muscle disharmony. However, electromyographic evidence of muscle balance in rest position could be obtained in more than half the patients in spite of occlusal interference and temporomandibular joint pain. Masticatory contacts were seldom observed posterior to centric occlusion. However, the occlusal contacts in swallowing apparently occurred between centric occlusion and centric relation. The occlusal stability in centric relation was found to be of extreme importance for the muscle harmony during swallowing. Rest position determined by conventional clinical methods did not always correspond electromyographically to a minimal harmonious muscle activity. A slight opening from the clinically determined rest position often facilitated the recording of an even minimal contraction of the temporal and masseter muscles. From this position the mandible could be opened about $\frac{1}{2}$ inch farther ("a resting range") without eliciting any increased muscle activity.

S21. Clinical Evaluation of Chewing Gum in Gingivitis and Dental Care.—*Patrick D. Toto, Gustav Rapp, and John O'Malley, Loyola University, Chicago.* The object of this study was to investigate the effect of chewing gum on caries and gingivitis of school-age children over a period of 1 year. Children from two primary parochial schools in Chicago were selected for this study. School 1 contained 275 children and served as the experimental group; school 2 contained 276 children and served as the control. The experimental group was given chewing gum with instructions to chew for 5 minutes after meals three times a day. This was supervised by the teachers in the school. The subjects were examined with mirror, exploring point, and bitewing radiographs at three periods: January, June, and December, 1956. The PMA was scored after examining the gingiva between the upper six anterior teeth. The mean DMF and PMA for each age group were recorded. The difference in means was calculated by using the first and third examinations. The mean increase of DMF in

both groups of school children is identical. This was calculated by securing the average of the sums of the differences of the means in each age group. The PMA shows a definite increase in the experimental group, while there was a decrease in the control groups.

School	Number	First Exam Mean		Third Exam Mean		Difference Mean	
		DMF	PMA	DMF	PMA	DMF	PMA
1 (Exp., gum)	275	4.7	4.4	7.1	7.75	2.4	3.3
2 (Cont., no gum)	276	4.5	2.2	6.9	1.3	2.4	0.4

S22. Cytochemical and Biochemical Studies of Respiration in Human Gingiva.—*Philip Person, S. Sigmund Stahl, and Sylvia Scapa, Veterans Administration Hospital, Brooklyn, New York.* Specimens of gingiva (clinically evaluated as slightly or non-inflamed) were obtained from 25 patients. Tissues were immediately chilled and frozen sections cut at 40 μ or at 100–120 μ. The 40 μ sections were incubated with a 1:1 mixture of alpha-naphthol and dimethylparaphenylenediamine, and the synthesis of indophenol blue (Nadi reaction) was followed. The thicker sections were homogenized in a ground-glass homogenizer, and O_2 uptakes of homogenates were measured with a standard manometric assay for cytochrome oxidase. With respect to indophenol blue production, the dye was first produced in basal cells of the non-keratinized gingival crevice epithelium. Second in activity were the basal cells of the cemental gingivae. Next, dye formation occurred in the outermost layers of epithelium. After 45 minutes to 1 hour, the keratin itself assumed a deep-blue coloration. Whether this was due to solution of the dye in lipids present in keratin or to an actual oxidation occurring within the keratin is not yet known. Axide and cyanide controls were used in the above experiments, and urethane was employed in some instances in an attempt to inhibit competing dehydrogenase systems. Oxygen uptakes ranged between 17 and 25 μl/hr/mg N. In some specimens, however, no oxygen uptake could be detected, and in one case a very low value, i.e., 5–6, was found. In many experiments oxygen uptake ceased after 60–90 minutes. These results suggested that endogenous inhibitors of oxidative metabolism might be present in the tissue. Preliminary evidence that this might be so was attained in several experiments in which gingival homogenates, added to heart-muscle cytochrome oxidase, inhibited oxygen uptake by the oxidase.

XXV. Abstracts of Papers Presented to the Dental Materials Group*

M1. Evaluation by Dye Penetration of Eastman 910 as a Cavity Liner.—*Barrie R. D. Gillings and Michael G. Buonocore, Eastman Dental Dispensary, Rochester, New York.* Eastman 910 (methyl 2-cyano-acrylate), an easily applied monomer which rapidly polymerizes to a clear, hard resin, was compared with zinc oxide-eugenol, oxyphosphate cement, and two commercial products† as a cavity liner. The performance of the liners was assessed by ability to prevent dye penetration of underlying dentin in both human and yearling bovine incisors, upon immersion in basic fuchsin solution. Liners were tested alone and in combination with silicate restorations, for varying time periods. Teeth were completely covered with wax except for the test area and were immersed both at constant temperature and with cycling (5°–50° C.). Penetration was recorded as none or slight if confined to the area immediately adjacent to the liner; moderate, if about halfway through the underlying dentin; and severe if reaching the pulp. Yearling bovine teeth presented greater uniformity in dentin permeability than did pooled human teeth, thus providing a less variable

* This meeting coincided with that of the International Association for Dental Research.

† Handi-Liner, Mizzy, Inc., and Cavaseal, Kerr Manufacturing Co.

measure of the effectiveness of the liner in limiting dye penetration. In both restored and unrestored cavities, the 910 liner was superior to both zinc oxide–eugenol and zinc phosphate cement. The latter materials showed extensive penetration within 1 week of immersion in nearly all cases, while 50 per cent of the 910-lined teeth showed little or no penetration after 6 weeks. Although the superiority of 910 over the proprietary liners was not so marked, better resistance to cycling, ease of application, and more desirable physical properties were noted.

M2. Permeability of Cavity Liners to Certain Agents.—*M. L. Swartz and R. W. Phillips, Indiana University School of Dentistry, Indianapolis.* A reduction in the solubility of enamel with an accompanying increase in fluorine content has been found after contact with silicate cement and other restorative materials containing fluorine. The purpose of this study was to determine whether cavity liners would alter the protective action of restorative materials by acting as a barrier to fluorine. The acid solubility of the untreated tooth surface was first measured, then a coat of varnish was applied and the restorative material placed over the varnish. At the end of the test period the materials were removed, and the solubility of the enamel surface was again determined. Where sodium fluoride solutions were studied, the varnish-protected teeth were merely immersed in the solutions. Three commercial cavity liners were included in the study. The passage of fluorine ions was indicated to some extent by the fact that when fluoride solutions, silicate cements, and resins containing fluorine were applied to enamel coated with varnishes, the solubility of the underlying enamel was reduced. However, the reduction was less than when silicates and aqueous sodium fluoride solutions were applied directly to the surfaces. The lining agents also retarded the action of acids. In some instances the amount of calcium dissolved from enamel surfaces protected by the varnishes was 80 per cent less than that dissolved from the unprotected controls.

M3. Fluorine Uptake by Intact Enamel from Certain Dental Materials.—*J. R. Platt and R. D. Norman, Indiana University School of Dentistry, Indianapolis.* As previously reported, the fluorine content of powdered enamel was altered by contact with various dental materials. This investigation was concerned with the effect on intact enamel. A layer of enamel of controlled depth was removed from half the labial tooth surface and analyzed for fluorine. This served as the control. The restorative material was applied to the remaining intact surface. At the end of the test period the enamel exposed to the test material was ground away and also analyzed for fluorine. The effect of silicates, restorative resins, and zinc phosphate cements was investigated. The effect of cavity liners was included in the study. Results were similar to those obtained with powdered enamel; however, the magnitudes were not of the same order. Generally, the changes were less than those noted with powdered enamel. Nevertheless, the increases in fluorine content of intact enamel exposed to restorative materials containing fluorine were most definite. The use of a cavity varnish tended to reduce the uptake of fluorine from these materials.

M4. Investigation of the Penetration of Cavity Liners by Phosphoric Acid.—*Wallace W. Johnson and James H. Wick, State University of Iowa, College of Dentistry, Iowa City.* Ten cavity liners or varnishes were selected as representative samples of the liners or varnishes that are currently being employed for protecting the dental pulp. Histologic, radiochemical, and dye-penetration methods of study were conducted on approximately 320 vital dog teeth and on 120 extracted human teeth. In the radiochemical study, penetration by phosphoric acid was investigated by utilizing the radioactive P_{32} isotope to tag the phosphoric acid of dental cement liquids. The relative amounts of penetration were determined by microscopic inspection and by radioautographic examination. Analysis of the findings revealed various degrees of penetration in each instance. Some of the liners consistently showed more penetration by the phosphoric acid than did the others.

M5. TOOTH FLUOROMETER.—*John W. Kumpula and Joseph J. Barone, National Bureau of Standards, Washington, D.C., and A. F. Forziati, American Dental Association, Research Division, National Bureau of Standards, Washington, D.C.* A fluorometer for the measurement of the fluorescence of teeth in the mouth has been constructed. The fluorometer consists of a medium-pressure mercury-vapor lamp (as a source of ultraviolet radiation), a filter passing only ultraviolet radiation in the 3650 A region of the spectrum, a lens for focusing the radiation onto the specimen, an annular lens to collect the emitted fluorescence, another filter (passing only visible radiation), and a photomultiplier radiation detector. The signal from the photomultiplier is fed into a direct current amplifier equipped with attenuation and sensitivity controls. Drawings and photographs of the instrument were shown. Calibration of the instrument, applications, and preliminary data were discussed.

M6. CUTTING STUDIES AT AIR-TURBINE SPEEDS.—*L. Kurlansik, J. O. Semmelman, and P. R. Kulp, Dentists' Supply Company of New York, York, Pennsylvania.* Tests were conducted of air-turbine handpieces operating under a range of air pressure and applied loads, measuring rotational speed and cutting effectiveness. Optimum cutting speed was found to vary with the hardness of the material being cut and to be virtually independent of power or torque. Cutting effectiveness was fairly stable at the optimum speed, and efficiency bore a generally inverse relationship to power input.

M7. TOOTH-PULP TEMPERATURE CHANGES PRODUCED BY CUTTING WITH AIR-TURBINE HANDPIECES.—*M. G. Wheatcroft, J. E. Hartnett, and W. F. Smith, U.S. Naval Dental School, National Naval Medical Center, Bethesda.* Temperature changes in the pulp were measured during cutting procedures using air-turbine handpieces. A copper and constantin thermocouple was placed in the pulp in contact with the dentin beneath the operation site. In some experiments an additional thermocouple was placed at the apex of the tooth to determine whether heat was transmitted to this area. A galvanometer* was used to show the temperature change. Cutting was performed on cuspids of living dogs and on freshly extracted human teeth. Number 558 carbide burs and diamond cylinders were used with air as the coolant. No increase in temperature was noted near the apex of the tooth. In all experiments no increase in temperature was observed until the cutting neared the thermocouple. The highest temperature increase observed was 9.5° C., and this occurred during rapid cutting. Considerable heat was produced at the cutting site but was not transmitted over any measurable distance. It appears that factors other than heat are responsible for pulpal changes when air urbines are used for cavity preparations.

M8. SURFACE CHARACTERISTICS OF TOOTH STRUCTURE AFTER CUTTING WITH ROTARY INSTRUMENTS.—*Kenneth R. Cantwell, Arthur W. Aplin, and David B. Mahler, University of Oregon Dental School, Portland.* The objective of this study was to compare the surface characteristics of tooth surfaces prepared by rotary instruments at varying speeds from 3,000 to 250,000 rpm so as to determine the best clinical method for finishing the enamel walls of a cavity preparation. The specimens for examination were recently extracted upper and lower anterior human teeth. The cuts were made at the incisal third of the labial surface and extended from the mesial to the distal proximal angles of the tooth. The handpiece was operated by hand, employing very light operating force with a sweeping brushlike action to duplicate clinical methods of finishing an enamel wall. The specimens were then cleaned and dried and shadow-cast under vacuum with aluminum. Photographs were taken and enlarged 10×. Crosscut-fissure carbide burs operating about 100,000 rpm produced the roughest surface; the plain-bladed burs left the smoothest surface. Crosscut-fissure burs operating between 3,000 and 35,000 rpm left a comparatively smooth surface. Diamond abrasive points produced a rough surface at all speeds. White polishing stones left a comparatively smooth surface above 100,000 rpm but resulted in a smoother surface when used below 100,000 rpm.

* Leeds-Northrup.

M9. Effect of Particle Thickness of Zinc and Non-Zinc Alloys.—*Malcolm D. Jendresen and Gunnar Ryge, Marquette University, Milwaukee, Wisconsin.* Two amalgam alloys of similar composition, except that one of the alloys contained 1 per cent zinc substituted for 0.7 per cent silver and 0.3 per cent tin, were fabricated under standardized conditions. Each alloy was cut by three different rates of feeding of the ingot to the milling tool, and for each cutting rate three different screening sections were separated. This procedure resulted in eighteen alloys, nine containing 1 per cent zinc and nine non-zinc alloys. Particle thickness determinations were carried out on all alloys; they were also tested for dimensional change, flow, and 1-hour compressive strength. On the basis of these tests, four alloys were selected for further testing; namely, one very fine-cut and one rather coarse-cut alloy of each of the zinc and non-zinc varieties. It was found that amalgam made from the zinc-containing alloys gave more favorable results in compressive strength tests, flow tests, and dimensional change determinations than did the zinc-free amalgam. Thicker particles resulted in lower early compressive strength values for both types of amalgam, with highest values recorded for amalgam containing zinc. Thicker alloy particles also resulted in amalgams of less contraction or more expansion. Amalgam made from zinc-containing alloys exhibited more dimensional stability than did the non-zinc amalgam.

M10. Amalgam Mercury/Alloy Ratio as Related to Change in Strength with Time.—*G. T. Mizera and E. W. Skinner, Northwestern University, Chicago.* The increase in strength as affected by amalgam mecury/alloy ratios from 0.33 to 2.20 has been studied for time periods (after condensation) ranging from 1 hour to 1 week. Five different commercial amalgam alloys were included in the tests. Although the strength of the amalgam from a given alloy appeared to be constant at the end of 1 week, regardless of the ratio employed, the rate of increase in strength with time less than 1 week was apparently related to the mercury/alloy ratios employed. With mercury/alloy ratios of 1.3 or higher, the maximum strength was attained in 8 hours. With lower ratios, the less mercury included, the longer was the time required to reach a strength maximum.

M11. Effect of Alloy-Mercury Ratio on the Physical Properties of Amalgam.—*W. T. Sweeney* and *Claire L. Burns, National Bureau of Standards, Washington, D.C.* Some physical properties of amalgams made from four brands of dental alloy were studied for alloy to mercury ratios from 1:1 to 1:10. The compressive strength, dimensional change on setting, flow, and residual mercury content were determined by standard methods. For alloy-mercury ratios ranging from the manufacturers' recommended values to one part alloy per ten parts of mercury, there was little observed effect on the compressive strength. Over this range of ratios the residual mercury content varied a maximum of 3 per cent for any one alloy. An additional study was made of the effect of strain rate on crushing strength, using 4×8-mm. cylindrical specimens. Varying head speeds of from 0.003 to 0.050 inch per minute produced crushing strengths ranging from 30,000 to 50,000 psi. These data indicate that the physical properties of amalgam are not significantly affected by the alloy-mercury ratio, provided that an essential minimum of mercury is present.

M12. Hardness Variations Found when Amalgam Was Joined.—*Herbert D. Ayers, Jr., School of Dental and Oral Surgery, Columbia University, New York.* Dental amaglam is sometimes placed in contract with part of previously placed amalgam fillings. The microstructure of the joints indicates that there may be wetting and solution of the residual filling material as well as faults which interrupt the junction. This study is concerned with the Knoop hardness variations that are found at, and adjacent to, such joints. The specimens were prepared in two steps. The first section was packed in a cylindrical plastic mold about 4.7 mm. in diameter and was allowed to set for more than 24 hours. The second part was then packed against the first in a

hole drilled for the purpose or in a recess left by a spacer that had been removed. After more than 24 hours the surface was polished by usual metallurgical methods. Knoop hardness indentations using a 10-gm. load were made in a line passing from within one mix across into the second. Photomicrographs showed the variation in size of indentation within the surface of controls and across the joined specimens. Etching was used to indicate the nature of hard and soft areas. Findings indicate substantial variation in hardness in the controls. Increased variation and lower Knoop hardnesses were found at, and adjacent to, the joint, disregarding indentations made on faults.

M13. Mechanical Failure of Amalgam Restorations with Different Underbase Materials.—*Duncan McConnell and David C. Hoppenstand, Ohio State University, Columbus.* Class I restorations of amalgam were implaced above bases of zinc phosphate and zinc oxide–eugenol in "Ivorine" teeth in order to ascertain comparative mechanical performances. The loads required to produce failure were applied to the occlusal surfaces by means of a $\frac{1}{8}$-inch steel ball. When the cavity preparation was shallow and the base material was confined to a small hemispherical excavation immediately above the position of the pulpal chamber, the strength with a zinc phosphate base was the same as that with amalgam alone. No significant reduction occurred when zinc oxide–eugenol was used. However, when the cavity preparation was deeper and when the base material formed a continuous layer on the floor of the cavity, the results were markedly different. An appreciable decline in strength was noted for zinc phosphate, and the decline was extreme for zinc oxide–eugenol. Even the strength of the amalgam alone was measurably less than that with the shallow cavity preparation.

M14. Model Technics for Analyzing Resistance Form of Amalgam Restorations.—*Louis G. Terkla, David B. Mahler, and Leonard N. Johnson, University of Oregon Dental School, Portland.* This paper is a report of work done in continuation of previous efforts as presented at the 1959 meeting of the Dental Materials Group. In analyzing the resistance form of amalgam restorations, two model technics were used. Loads to produce fracture were applied to (1) amalgam placed in an actual-size metal model of a tooth and (2) plaster placed in an 8 × actual-size densite model of a tooth. These fracture loads were compared with loads necessary to produce fracture of amalgam in natural teeth. The data presented illustrate the difficulties encountered in working with model technics of stress analysis and the steps used to correct these difficulties. At this time, the fracture of plaster appears more sensitive to design variations than does amalgam when placed in either natural or metal teeth. Present data also indicate that minor design variations have no significant effect on the fracture of amalgam.

M15. Anticariogenic Effect of Silver Amalgams and Gold Inlays in Vivo.*—*O. D. Harvey, Emory University School of Dentistry.* This paper is concerned with a portion of the information compiled in a 2-year clinical survey at Emory University School of Dentistry. An effort was made to associate known in vitro findings of the anticariogenic effect of silver amalgam and gold inlays with the final data of our in vivo study. In our study, we tried to demonstrate how effectively silver amalgam and gold inlays appeared to resist recurrent caries at their margins. The restorations were all inserted in the clinics of the School of Dentistry, and complete records were available. By use of these records, age of patient and age of restoration could be determined at the time of the survey. Restorations were checked in the selected mesio-occlusal areas of the first molars and the disto-occlusal of the second bicuspids in each arch. For this and other information, records of over 15,000 patients were sorted. Fifteen hundred patients were called, and, of these, 415 came to the dental clinic for examination.

* This investigation was supported in part by Research Grant D-426 from the N.I.D.R., U.S. Public Health Service. Statistical data computed by Mr. Clint Terrell, Georga State Health Department statistician.

Examinations were made by three examiners. 1,197 restorations of 395 patients were checked as acceptable, and, of these 1,197 amalgams and inlays, 99 were failures due to recurrent decay for 4.9 per cent. Of 531 amalgams, 33 failed because of recurrent decay for 6.2 per cent. Of 666 gold inlays, 26 failed for 3.9 per cent.

M16. Study of Silver-impregnated Calcium Hydroxide in Pulp Capping.—*Harry B. Sheldon, Georgetown University School of Dentistry, Washington, D.C.* Calcium hydroxide is available in a commercial preparation, in paste form, for use in the capping of dental pulps. Many investigators have proved that this material stimulates secondary dentin formation, neutralizes the free acid in dental cements, and prevents thermal shock. The American Dental Association Council on Accepted Dental Remedies placed calcium hydroxide methyl cellulose paste in Group A in March, 1955. The widespread use of this material by great numbers of practicing dentists has brought about a severe problem, that of distinguishing calcium hydroxide from carious dentin beneath existing restorations in radiographs. A survey of over one hundred dentists showed that they could not distinguish caries from calcium hydroxide in such films. This project, by the use of readily available commercial products, illustrates a dental material that is easy to use and makes the problem of distinguishing caries from calcium hydroxide in a radiograph a simple matter. The addition of one part precipitated silver to six parts of calcium hydroxide methyl cellulose paste by weight did not affect the apparent chemical composition, physical properties, or shelf age. In the clinical and roentgenologic investigation of over two hundred cases capped with silver-impregnated calcium hydroxide methyl cellulose paste, there was no decrease in the effectiveness of the treatment when compared with teeth treated with the approved calcium hydroxide methyl cellulose paste.

M17. Relationship between Retaining Force of Inlays and Film Thickness of Zinc Oxyphosphate Cement.—*Takao Fusayama and Tsugio Iwamoto, Tokyo Medical and Dental University, Tokyo.* The relationship between pressure and film thickness was first investigated by cementing two square metal plates of $(5\ mm.)^2$ area with a zinc oxyphosphate cement. The results were described by hyperbolic curves. A thickness of 35–40 μ at a load of 10 gm. produced the curve that reached the point nearest 0. Plate-form metal inlays having a $(5\ mm.)^2$ cementing area were cemented on either enamel, dentin, or metal surfaces with different cement thicknesses caused from various loads and different temperatures. The shearing resistance of cementation of these specimens was measured with a special apparatus designed by the authors. Separation was usually found at the metal-cement junction. The cement thickness which best tolerated a maximum force for retaining inlays was 38 μ for enamel, 31 μ for dentin, and 24 μ for metal surface. More than 60 μ produced maximum force on wet dentin. The retaining force of inlays with a given cement thickness was increased at higher temperatures. A greater force could be exerted on enamel than on dentin, and very little force could be exerted on wet dentin. Drying dentin, beyond that found under normal clinical conditions, slightly increased the retaining force according to the shearing test.

M18. Dimensional Stability of Denture-Base Resins. I. Clinical Evaluation.—*F. D. Mirza, Northwestern University, Chicago.* Thirty-three maxillary and mandibular clinical dentures were constructed in acrylic resin via the standard monomer-polymer dough technic and heat-cured. After insertion, these dentures decreased continually in linear dimension from molar to molar region for approximately 1 week. At 1 month they showed a slight expansion; this expansion, however, failed to compensate for the curing shrinkage. At 3 months very little dimensional change was noted. A second group, consisting of thirty-one upper and lower dentures, utilized an autopolymer acrylic resin.* These dentures were prepared via a casting technic in a flexible mold. After gelling at room temperature they were post-cured 30 minutes

* Acrylcast.

at 72° ± 2° C. in a dry-heat oven. The average processing shrinkage of both groups was approximately the same. The cold-cured denture group continued to shrink throughout the entire testing period of 3 months. A statistically significant difference in the linear change between the two groups was noted at this time, the heat-cured group showing less shrinkage. After 3 months, all dentures were replaced on the initial models. Perceptible changes were evident, but no correlation was found with the clinical fit. On the basis of patient reaction and clinical observation, the amount of linear change did not significantly affect the fit of the dentures of either group. The anterior-posterior linear changes were also recorded and were found to vary greatly from one denture to another in both groups. These data could not be analyzed meaningfully.

M19. Dimensional Stability of Denture-Base Resins. II. Laboratory Evaluation.—*L. A. Landry, Austenal Company, F. D. Mirza, Northwestern University, Chicago, and P. H. Erdle, Austenal Company.* The dimensional changes occurring in four denture-base resins upon prolonged immersion in 37° C. water were studied. Two standard acrylic resins were processed via the monomer-polymer-dough technic and heat-cured; one acrylic resin was processed via a casting technic, allowed to cure at room temperature, and then post-cured for various lengths of time. One experimental epoxy resin was processed via a casting technic and heat-cured. Palate fit was followed on denture blanks; intertuberosity change was followed on full upper dentures. The epoxy resin was best regarding both processing accuracy and stability of palate fit. Intertuberosity change after processing was —0.4 per cent and changed linearly with time to a value of +0.04 per cent after 7 months. The heat-cured acrylic resins showed a processing shrinkage from 0.4 to 0.5 per cent. The shrinkage increased for a short time and then leveled off to 0.35 per cent. Use of a cross-linking monomer lowered the processing error and increased the stability of the palate fit. The cold-cure casting system using the improved autopolymer acrylic resin* (utilizing a special cross-linking monomer) was evaluated at various processing cycles. Intertuberosity change varied from —0.3 to —0.5 per cent, depending on post-cure (increasing negatively with increasing post-cure), and leveled off more or less after 1 month at — 0.15 to — 0.3 per cent. Factors influencing dimensional stability were (1) the nature of the polymer, (2) the degree of cure, (3) cross-linking density, (4) the presence of teeth and varying resin thickness throughout a denture, and (5) the nature of the sealer. Also there was a correlation between the rates of water absorption and dimensional change of the resins studied.

M20. Dimensional Changes in Artificial Dentures on Drying, Wetting, and Heating in Water.—*George C. Paffenbarger, American Dental Association Research Division, National Bureau of Standards, Washington, D.C., and Julian B. Woelfel, College of Dentistry, Ohio State University, Columbus.* Thin upper and lower dentures of eight types of acrylic resins and one each of polystyrene, vinyl-acrylic resin, epoxy resin, and vulcanite were (A) dried 22 days, (B) placed in water at 37° C. for 22 days, and (C) heated in water for 30 minutes at 10° intervals from 50° to 100° C. The weight loss in A ranged from 0.38 per cent (vinyl-acrylic resin) to 1.29 per cent (cold-curing acrylic resin); the weight gain in B ranged from 0.35 per cent (vinyl-acrylic resin) to 1.20 per cent (powder-liquid acrylic resin, Hydro-Cast technic). The gain in weight from B through C ranged from 0.34 per cent (cold-curing acrylic resin) to 0.14 per cent (polystyrene). On procedures A and B the dimensional changes generally followed the weight changes except that vulcanite expanded on drying and shrank on wetting. The average molar-to-molar and flange-to-flange changes on the sets of dentures in A ranged from —0.84 per cent (powder-liquid acrylic resin) to +0.30 per cent (vulcanite); in B, from +0.83 per cent (powder-liquid acrylic resin) to —0.20 per cent (vulcanite). In C the severest distor-

* Acrylcast.

tions occurred in the polystyrene dentures and the least in the cold-curing acrylic resin. The temperature at which the major part of the strain was relieved ran from 50° (glass-fiber-filled acrylic resin) to 90° C. (polystyrene).

M21. Changes in the Relative Positions of Teeth in the Construction of Denture Bases.—*D. F. Vierira, Indiana University School of Dentistry, Indianapolis.* In the laboratory phases of the construction of a denture base there are some changes in the position of the teeth modifying the occlusion previously obtained. Reference points were inserted in several teeth, and these alteration changes during storing of base-plate wax dentures, curing of the denture base, and consequent finishing and polishing of the same were studied. The distances were measured with the aid of a comparator microscope. The following results were obtained: (1) the storing of the base-plate wax denture was a cause of change in the teeth position; (2) the distances changed differently according to the material used (acrylic, acrylic and Perlon mash, polyamide); (3) the changes differed between upper and lower dentures; (4) the setting expansion of the stone used to invest the dentures, as well as the Perlon mash, had no influence on the changes found; (5) the changes found with polyamide were greater than those with acrylic resin; (6) the form of denture was an influential factor—upper dentures showed different changes when compared with the lower one; and (7) during the finishing and polishing of the denture there was a change in the relative position of the teeth.

M22. The Dimensional Accuracy of Denture Base Materials.—*D. H. Anthony and F. A. Peyton, University of Michigan, Ann Arbor.* The contours of dentures made of various materials and of the master impression were recorded in graph form as a result of measurements with a modified pantographic device, or comparator. Although there were minor variations in contour, the dentures of different types of materials could be arranged in well-defined groups. After 24 hours' storage in water, the self-cured group showed excellent accuracy of fit; the heat-cured group showed somewhat less accurate fit; and the special group, processed by both heat and injection, showed the least degree of accuracy. In general, the same pattern of fit occurred with all these plastic dentures. The shrinkage in the upper dentures caused the flanges to be drawn inward in varying amounts and resulted in greater or lesser discrepancies in the palatal region. Shrinkage in the lower dentures resulted in the lingual borders pulling away from the model. Other materials, such as cast-metal bases, porcelain, vulcanite, and an epoxy product were also measured and evaluated. After 8 months' storage in water the slight expansion which occurred in most of the plastic dentures allowed a further seating on the model and an improved over-all fit.

M23. Application of Miniaturized Electronic Devices to the Study of Tooth Contact.—*Allen A. Brewer and Donald C. Hudson, School of Aviation Medicine, Brooks Air Force Base, Texas.* An entirely self-contained radio transmitter, fabricated of miniature components, was built into the base of several sets of complete dentures. Output was controlled by contact of the posterior teeth, which were of the gold occlusal type. There were no wires or other projections on the dentures which would interfere with their normal use. Signals generated upon contact of the teeth were picked up by an external antenna in the shirt collar or taped to the neck of the subject. Records of the number of tooth contacts during chewing, at rest, or during normal daily activity were made by means of a portable counting device. Laboratory recording was done on an electrocardiograph. A limited number of subjects wearing complete dentures were studied, using the methods outlined, to determine the effect on tooth contact produced when various cusp heights, occlusal forms, or tooth arrangements are employed. Further miniaturization is being attempted in an effort to produce a transmitter small enough to be placed in partial dentures of either removable or fixed type.

M24. Thermal Expansion of Denture Base Materials.—*Ruth M. Davenport and George Dickson, National Bureau of Standards, Washington, D.C.* Thermal expansion measurements were made on ten denture base materials and one sample of commercial Lucite rod. Measurements were made over a temperature range of approximately 20°–70° C. with the fused-quartz tube apparatus. The materials were run dry and then wet, after having been stored in water at either 68° or 37° C. for several days. The dimensions of the molded specimens were approximately $8 \times \frac{7}{16} \times \frac{7}{16}$ inches. The results show that coefficients of thermal expansion of the methyl methacrylate denture base resins averaged slightly higher over the 27°–67° C. range than the approximately 90×10^{-6} per degree obtained for commercial Lucite rod. The coefficients for vinyl copolymer, styrene, and epoxy denture resins were approximately 10, 20, and 25 per cent below the average for the methyl methacrylate materials. Methyl methacrylate resins containing large amounts of glass had coefficients over 50 per cent lower than those of the other methyl methacrylates. Soaking in water at elevated temperatures for several days raised the average coefficient of methacrylate materials by approximately 10–15 per cent.

M25. Pyrolysis Products of Acrylic Resins.—*Frank A. Lehmann and G. M. Brauer, National Bureau of Standards, Washington, D.C., and A. F. Forziati, American Dental Association Research Division, National Bureau of Standards, Washington, D.C.* Pyrolysis of methyl methacrylate and selected acrylic resins was studied at various temperatures. A small boat* surrounded by a heating coil† was placed in the inlet system of a gas chromatograph. The 5–10-mg. sample was degraded at a constant temperature, which was measured by means of a thermocouple placed in an indentation at the bottom of the boat. A regulated flow of helium carried the volatile pyrolysis products into the heated column of achromatograph. Changes in the degradation temperature produced different pyrolysis products, as indicated by the appearance of new peaks on the chromatographs. Poly (methyl methacrylate) degraded almost exclusively into monomer at temperatures ranging from 350° to 600° C. At higher temperatures, at least seven distinct products could be observed. Collection of individual products and subsequent identification by infrared spectrophotometry or mass spectrometry appear feasible. The method rapidly detects the presence of different pyrolysis products and aids in their identification. It readily detects the thermal stability of polymers and assists in the elucidation of their mechanism of degradation over wide temperature ranges. The procedure may also prove useful in the characterization of complex polymer systems.

M26. Comparison Studies of the Retentive Ability of Temporary Cements.—*Edward G. Kaufman, Laurence Colin, and David H. Coelho, New York University College of Dentistry, New York.* It was the purpose of this study to re-evaluate the retentive ability of the zinc oxide–eugenol cements as compared with the zinc phosphate cements. It has been reported that zinc phosphate cement as a cementing medium has poor flow characteristics, is relatively soluble in certain media, and produces pulpal irritation to a greater degree than do the zinc oxide–eugenol cements. In this study aluminum alloy dies and crowns were machined and fitted within a tolerance of 0.0002 inch. Extensions were provided on both the crowns and the dies so that the cemented assembly could be placed in the jaws of a tensile tester and a load applied. The crowns were cemented to the dies under controlled conditions, using several zinc oxide–eugenol cements, and the forces required to unseat the crowns were determined. From a comparison of these data with those obtained from similar studies using zinc phosphate cement, it appeared that most of the temporary type cements exhibited more than half the retentive ability of zinc phosphate cement, with the exception of one which closely approximated the zinc phosphate values. Further studies are indicated to determine the actual range of retention required in cementation procedures and in the development of a zinc oxide–eugenol cement that meets this requirement.

* Vycor. † Nichrome.

M27. Improved Cementation.—*Harold R. Horn, Department of Denture Prosthesis, New York University College of Dentistry, New York.* Zinc oxide–eugenol cement mixtures are so effective in their therapeutic action on the dental pulp that much of our present-day research is involved with improving this material for use in permanent cementation as well as in temporary cementation of fixed prosthesis. The only characteristic of a good permanent dental cement that a zinc oxide–eugenol mixture lacks is hardness of set. This quality may be added to a zinc oxide–eugenol mixture by refining and decreasing the particle size of the zinc oxide powder, by incorporating zinc acetate into the mix, by incorporating dicalcium phosphate into the mix, by the addition of polystyrenes to the eugenol, and by the addition of various chelating agents.

A preliminary evaluation of a new material, Triamite, suggests further improvement to zinc oxide–eugenol mixtures in temporary and in permanent cementation. Triamite is an organic derivative of a substituted benzoic acid and, when incorporated with any cement mixture in the small amount of 1 per cent, will give to that cement greater strength, greater bacteriostatic properties, and a more homogeneous mix. The foul, nauseating smell associated with the removal of temporarily cemented restorations is eliminated by incorporating a small amount of Triamite in the mix. The addition of a small amount of Triamite to any cement mixture results in a liquefying phenomenon that enables the incorporation of additional powder, resulting in a cement mix of greater strength. Triamite contributes germicidal properties to the cement mixture.

M28. Effective Setting Expansion of Investment in Casting Rings.—*A. Bruce Ady and David B. Mahler, University of Oregon Dental School, Portland.* The expansion potential of an inlay casting investment is determined by many factors which include composition, condition of the investment, water to powder ratio, and spatulation conditions. This potential is not completely realized under practical conditions because of the restrictive influence of the wax pattern and the casting ring. This expansion, appropriately termed "effective" setting expansion, which actually results in a change in the size of the mold cavity formed by the wax pattern, was investigated in this study. Wax patterns were made in a steel die having precise measuring points. These points which were reproduced in the wax patterns were measured, and the patterns were then invested, using several investing technics. After set, the investment was trimmed and the wax pattern picked out. The investment was then measured to these same points. Using an improved stone as a control test, the accuracy of measurement was determined as ± 0.1 per cent. For the various technics studied, the following number of test runs out of a total of 30 fell within the range of the arithmetic mean $\pm$ 0.2 per cent: thermal technic, 22 runs; hygroscopic, water-bath technic, 16 runs; hygroscopic, water-added technic, 28 runs. These results demonstrate that control of the hygroscopic effect is essential in reproducing investment setting expansion within significant limits.

M29. The Effect of the Asbestos Liner of the Investment Mold.—*D. W. Palmer, A. F. Romnes, and E. W. Skinner, Northwestern University, Chicago.* A 1-cm. invar steel cube was invested in an inlay ring in the usual manner except that the length of the asbestos liner in the ring was varied. After set, the investment was sectioned so that one face of the cube was exposed. The spaces between the investment and the four faces perpendicular to the exposed cube face were carefully measured and compared as to magnitude laterally and longitudinally with respect to the casting ring. Since the dimensions of the cube were known, the percentage setting expansion could be determined in relation to the change in the mold size. When no liner was used, no setting expansion was observed. When the liner was present, the lateral setting expansion was the same regardless of the length of the liner. The expansion longitudinally was greater than the lateral expansion when the liner extended

the full length of the casting ring. When it was shortened $\frac{1}{8}$ inch equally from each end of the ring, the longitudinal expansion was less than the lateral expansion.

M30. The Effect of Longitudinal Restrictive Force on Hygroscopic Expansion.—*John S. Shell, Ransom & Randolph Company, Toledo, Ohio.* Force applied on investment during setting reduces the setting expansion, as has been shown by Skinner. It also reduces the hygroscopic setting expansion, as originally indicated by Scheu. Force also delays the initiation of hygroscopic expansion. An attempt was made to apply sufficient force during hygroscopic expansion to reduce these quantities to the amount obtained during normal setting expansion. Forces up to 1 atm. were almost sufficient to cause this amount of reduction in expansion. The per cent reduction in both normal setting expansion and hygroscopic expansion was determined in a range of 0–1,000 gm/cm^2, using a specimen of 2.5 cm. in cross-section by 10 cm. in length, lined on three sides with asbestos and covered with water for 2 hours. A balanced dial gauge was used. These expansions were compared with those obtained by using a comparator microscope and wax-paper–lined trough, as well as with the wire method using a calibrated micrometer eyepiece. The wire method often indicated hygroscopic expansions approximately twice those obtained by the trough method. Reduction in the horizontal longitudinal hygroscopic expansion resulting from force on the specimen in the direction of measurement also produced reduction of expansion in a vertical direction.

M31. A Calorimetric Study of the Hygroscopic Setting of Calcined Gypsum.—*Joan A. Donnison, M. P. Chong, and A. R. Docking, Commonwealth Bureau of Dental Standards, Melbourne, Australia.* Calorimetric studies on the setting of gypsum products were carried out chiefly to determine whether there was any increase in the rate of evolution of heat when a sample was allowed to set under hygroscopic conditions. It was found that the rate of evolution of heat was greater when Hydrocal set under water rather than under an inert liquid. It was also greater when extra water was added shortly after the commencement of mixing, the total amount of heat evolved at the end of the experiment, as well as the rate, being dependent on the amount of water added. By chemical analysis it was found that when sufficient water was added shortly after the commencement of mixing, any further addition of water did not effect more hydration. Also, the higher the water-powder ratio, the more water there was available and the smaller the effect of added water. The results indicated that hygroscopic setting is an extension of the setting process whereby hydration is completed to a greater degree.

M32. A Method for Evaluation of the Expansion of Casting Investment under Actual Working Conditions.—*George M. Hollenback, and John E. Rhoads (independent dental research).* It has long been felt that the measurement of the outside dimensions of test samples of casting investment is not necessarily a correct representation of what actually happens inside the mold cavity, either during the setting of the investment or during the subsequent mold-conditioning process. Because of this, it was felt that the possibility of actual mold measurement should be explored. Attempts to determine changes taking place in the mold cavity have been made by elaborate methods of measuring changes in the wax pattern itself (which cannot give a true picture of changes in the mold wall) and by the circuitous route of attempting to analyze dimensions of resultant castings made into the molds. Each of these methods, because an attempt is made to observe a primary change by means of a secondary result, may not show accurately the actual events taking place. A method of measuring has been developed whereby the dimensions of the mold cavity can be measured accurately during the period from initial set to final set of the investment. The method is simple, straightforward, and not subject to great experimental error. Results obtained using this equipment indicate that values considerably different from those previously established by currently used equipment do exist. Representative results

obtained in two series of three consecutive tests are shown in Tables 1 and 2, which illustrate the comparison between the measurement of the external dimension of specimens and measurement of the mold produced by the same mix of investment. Note the increased expansion afforded by the wet asbestos liner. It will be observed that results obtained by measurement of the mold cavity appear to show approximately half the expansion of that indicated by external measurement.

TABLE 1

MATERIAL: KERR CRISTOBALITE. BATCH 931 RA 2323. W/P 36/100; WET ASBESTOS LINER. TIME: 2 HOURS SET

Test	External Measurement (Per Cent)	Mold Measurement (Per Cent)
1	1.77	0.8
2	1.56	0.8
3	1.41	0.8
Average	1.48	0.8

TABLE 2

MATERIAL: KERR CRISTOBALITE. BATCH 931 RA 2323. W/P 36/100; DRY ASBESTOS LINER. TIME: 2 HOURS SET

Test	External Measurement (Per Cent)	Mold Measurement (Per Cent)
1	1.36	0.64
2	1.34	0.60
3	1.46	0.60
Average	1.39	0.61

M33. PRELIMINARY REPORT ON MICROMEASUREMENT FOR DETERMINING ACCURACY OF INLAY CASTINGS.—*E. A. Sahs and J. H. Wick, College of Dentistry, State University of Iowa, Iowa City.* It is often presumed that there is very little research left to be done in the field of metal castings. Such is not the case, and the final word has not been written on precision for dental castings. It is the hope of the investigator to compare technics with technics which manufacturers purport to be foolproof and thereby to determine the volume of space existing between the cavity walls and the casting as an indication of fit. The first phase of this project was the standardization of wax-pattern procedure. Wax patterns of cavities prepared in metal dies were taken at room temperature. On the basis of manufacturers' data the second phase of the project was concerned with the investing, burn-out, and casting. Eleven different investments were categorized, and base lines were established for controls. The third phase dealt with determining the volume of space between the casting and the walls of the cavity. Five materials—mercury, basic orthophosphoric acid, glycerin, light mineral oil, and dibutyl phthalate—were tested because they ran the gamut of the range of high to low surface-tension materials. The method used to determine the volume of space was the straight displacement method, and the volume of the materials was computed from the microweight and specific gravity. More research is necessary to establish an ideal fit and cementing media.

M34. Effects of Storage Conditions on Compressive Properties of Dental Gypsum.—*Carl W. Fairhurst, Marquette University, Milwaukee, Wisconsin.* The purpose of this investigation was to study the effects of storage media and storage temperature on the compressive strength of dental stone. A flow test was devised to measure the compressive flow of wet and dry gypsum. It was found that a continuous increase in strength occurred for both wet and dry stone as the water/stone ratio used for mixing decreased. Tests in which the quantity of excess water absorbed by dry stone was measured revealed that 0.1–0.2 per cent water absorption resulted in a 25 per cent loss of compressive strength. Dry stone immersed in 100 per cent ethyl alcohol or in transformer oil exhibited strengths equal to dry stone. Stone specimens stored in air at 65°, 75°, 85°, and 100° C. all exhibited compressive strengths lower than dry strength after 24 hours. The specimens stored at 100° C. lost 67 per cent compressive strength after 5 hours. X-ray diffraction studies showed that the gypsum specimens had been converted to hemihydrate during storage in air at these temperatures. Preliminary flow data show that dry stone exhibited nearly no flow at loads below the ultimate compressive load, whereas wet stone exhibited more than 1 per cent flow in 2 hours at loads equal to 83 per cent of the ultimate compressive strength, and more than 4 per cent flow in 72 hours at loads equal to 51 per cent of the ultimate compressive strength. A tentative explanation is proposed for these differences in wet and dry stone. The explanation is based on the precipitation of gypsum in the intercrystal boundaries of the hardened gypsum.

M35. Inherent Surface Roughness of Dental Gold-Alloy Castings.—*Joseph J. Barone and Richard L. Huff, National Bureau of Standards, Washington, D.C.* The surface roughness was measured on a series of 273 dental gold castings, using three different alloys cast with burn-out temperatures varying from approximately 800° to 1,600° F. and alloy casting temperatures varying within the vicinity of 1,725°–2,400° F. Extremely high mold and metal temperatures appeared to increase surface roughness, irrespective of alloy used. At burn-out temperatures to approximately 1,300° F. and metal casting temperatures to approximately 2,000° F., surface roughness appeared to be constant.

M36. An Investigation of Ultrasonics for Cleaning of Instruments and Processing Appliances.—*Gerald T. Charbeneau, University of Michigan, Ann Arbor.* Since the use of ultrasonic vibrations is becoming increasingly popular in industry and elsewhere for the removal of many types of surface contaminants and in various finishing processes, it appeared desirable to explain the potential applications of such equipment in dentistry. Two ultrasonic units, one operating at 40 kc. and the other at 50 kc., were employed in a variety of cleaning applications and laboratory procedures. The cleansing of many contaminated surfaces with this equipment was extremely rapid and efficient. The cleaning action was dependent on (1) the contaminant, (2) the type of surface from which removal was desired, (3) the cleaning agent, (4) the frequency of vibration, and (5) the time the object was subjected to the ultrasonic cavitation. The usual operating-room contaminants were removed from dental and rotating cutting instruments in approximately 2 minutes. The contaminant included combinations of dried blood and mucous, dried cut tooth tissue, and set dental cements. Gypsum-bound investments were readily removed from dental castings. Dental stones were, for the most part, removed from resin dentures in times ranging from 5 to 20 minutes. The ultrasonic cleaning unit has been found advantageous for the general maintenance of various types of dental and scientific equipment. The use of ultrasonic vibrations in dental electroforming and electropolishing was also briefly explored.

M37. The Effect of Remelting on the Mechanical Properties of Chromium-Cobalt Alloy.—*Duane F. Taylor, National Bureau of Standards, Washington, D.C., and Jay G. Hanson, Central Dental Laboratory, Walter Reed Army Medical*

Center, Washington, D.C. The reuse of previously cast metal is routine practice in the case of dental gold castings. However, the recommendations of various manufacturers of chromium-cobalt dental alloys differ greatly in this regard, some permitting as much as two-thirds scrap for any melt, while others advise the use of new metal only. A study was made to determine the importance of this factor under conditions of careful control and modern laboratory technics. An alloy was selected for which the use of new metal only is recommended by the manufacturer and which thus is presumably sensitive to the effects of remelting. The tensile properties of specimens containing metal melted and recast up to eight times were studied and compared with the properties of the new metal. Decreases in tensile strength, yield strength, and elongation were found in amounts varying with the number of meltings. In most cases even the maximum number of remelts did not lower the properties below the requirements of A.D.A. Specification No. 14. The clinical significance of these changes is being evaluated in a series of clinical partial-denture cases.

M38. The Flow and Fracture of Dental Alloys Determined by New Microbend Tester.—*Kamal Asgar, University of Michigan, Ann Arbor.* It has been shown that the microstructure of an alloy is the fundamental variable which controls its mechanical properties. Other simple tests, such as hardness or tensile strength, however, are commonly used to determine the mechanical properties of alloys. Usually a group of tensile specimens, after being tested, are polished and etched; then their microstructures are studied and correlated with their performance. With a microbend tester the microstructure of the alloy remains under observation as the specimen is stessed. In this way the exact behavior of the alloy, as well as the difference in the behavior of different phases of each alloy, can be observed. Specimens of dental gold alloys and cobalt-base alloys were made, and the behavior of their different phases under stress were studied. The most brittle phase in each case was observed. In this study it was shown which one of the phases of a particular alloy initiates the fracture and how the fracture proceeds.

M39. Electrochemical Phenomena at a Solid-State Junction.—*Saul M. Bien and Herbert D. Ayers, Jr., Columbia University, New York.* The junction of austenitic steel alloy with Ag-Cu-Zn-Ni solder alloy is the site of a sequence of electrochemical phenomena. In previous papers it has been demonstrated that irregular areas of varying concentrations of flux residue labeled with fluorine-18 were deposited at this junction. After 1 year of exposure to the room atmosphere in the laboratory, discoloration was observed (at $\times 375$) on the polished surface of the cross-sections of the solder joints on areas corresponding approximately to those which produced radiopacities on the autoradiographs. The surface was repolished, removing all stains. After 6 months of re-exposure to the air in the laboratory, discoloration again reappeared in the same areas of the surface adjacent to the interface. There appeared to be a correlation between the areas of the autoradiographs showing the deposition of fluoride salts at the interface and the discolored areas observed on the surface of the cross-section of the solder joints. The discoloration appeared to result from corrosive action in the areas which were most radioactive. The dissociation of the salts deposited at the interface between the solder and the chromium alloy with the movement of ions over the polished surface of the specimen may have been facilitated by moisture from the atmosphere. It is postulated that crevice attack is initiated by solution through diffusion at the interface. Following this, the crevice continues with the formation of an active-passive cell, a differential-aeration cell, or a differential metal-ion concentration mechanism.

M40. Properties of Inlay Casting Waxes.—*John W. Stanford, Keith V. Weigel, and George C. Paffenbarger, Research Division, American Dental Association, National Bureau of Standards, Washington, D.C.* To provide a basis for revision of American Dental Association Specification No. 4 for Dental Inlay Casting Wax, data

were obtained on the non-volatile residue, flow at various temperatures, and thermal expansions of eight waxes. The values determined for residue of eight waxes ranged between 0.00 and 0.08 per cent. The proposed maximum is 0.10 per cent. The values determined for flow at 37° C. for Type I waxes (those intended for direct technic application) ranged between 0.3 and 0.4 per cent. The proposed maximum is 1.0 per cent. Flow at 40° C. for Type I waxes ranged between 1.4 and 15.4 per cent, with a maximum of 20 per cent proposed. Flow at 45° C. for Type I waxes ranged between 74.5 and 77.2 per cent, with a minimum of 70 and a maximum of 90 per cent proposed. Flow at 30° C. for Type II waxes (those intended for indirect application) was 0.2 per cent, with a maximum of 1.0 per cent proposed. Flow at 40° C. for Type II waxes ranged between 54.1 and 77.4 per cent, with a minimum of 50 per cent proposed, and at 45° C. flow ranged between 79.0 and 85.2 per cent, with a minimum of 70 and a maximum of 90 per cent proposed. Linear thermal expansion of the Type I waxes between 25° and 30° C. ranged between 0.13 and 0.16 per cent, with a maximum of 0.2 per cent proposed. From 25° to 37° C. the expansion was between 0.34 and 0.50 per cent, with a maximum of 0.6 per cent proposed. Annealing of test specimens at 37° C. before testing is recommended.

M41. AN IMPACT TEST FOR DENTAL PORCELAIN.—*J. E. Fox, P. R. Kulp, and P. W. Lee, Dentists' Supply Company of New York, York, Pennsylvania.* Various impact tests from other fields were found to be unrelated to the clinical behavior of dental porcelain. A test was developed which correlated qualitatively with observed clinical behavior. It was based upon a weight falling from increasing elevations upon a symmetrically shaped porcelain specimen. Representative impact forces necessary for breakage included early opaque dental porcelain, 280 gm/cm; translucent enamel porcelain air-fired, 245 gm/cm; translucent enamel porcelain vacuum-fired, 420 gm/cm; translucent dentin porcelain vacuum-fired, 490 gm/cm.

M42. MICROINDENTATION HARDNESS.—*Donald E. Foley, Carl W. Fairhurst, and Gunnar Ryge, Marquette University, Milwaukee, Wisconsin.* The purpose of this investigation was to evaluate load dependence of microindentation hardness for enamel and dentin and for various dental materials, using the Knoop and Vickers indentors. Microindentation hardness was determined for enamel and dentin and for eleven materials (metals, cements, resin), using loads loads of 1, 5, 10, 25, 50, 100, 500, 1,000, and 10,000 gm., with constant rate of descent and constant dwell time. Specimen preparation was carried out under uniform conditions. The data were analyzed for compliance with the relation $P = ad^n$, and the exponent n was evaluated as a function of load. It was found that the hardness for most materials tested was load-dependent for low loads. In most instances a maximum hardness number was recorded for loads of 50, 100, or 500 gm. The exponent n in the relationship $P = ad^n$ did not exhibit systematic variations at low loads, as claimed by other investigators.

M43. STRUCTURAL DENSITY OF COMPACTED GOLD FOIL AND MAT GOLD.—*J. Turnbaugh Hodson and Gerald D. Stibbs, University of Washington School of Dentistry, Portland.* A method was developed from metallographic technics for determining the hardness density of compacted gold foil and mat gold. The procedure included the use of lucite mounts, hardness tester, and metallographic microscope. Microscopic structures were correlated with compaction and hardness.

M44. ANNEALING TEMPERATURE OF GOLD FOIL.—*P. H. Spencer, E. W. Skinner, and A. F. Romnes, Northwestern University, Chicago.* Ropes of dental gold foil were cut into lengths of average weight 74 mg. Each piece of foil was annealed for 3 minutes at a given temperature and immediately condensed in a "cavity" prepared in an Ivorene block, with the use of a high-frequency (200–3,600 v.p.m.) electromallet. Annealing temperatures ranging from 26° C. (room temperature) to 450° C. were employed. The cohesion obtained was measured by means of the Brinell hardness number. In general, the hardness at the edge of the specimen was less than that at the

center. The lower the annealing temperature, the greater was the hardness when a low intensity (setting 2) and the maximum frequency were employed. With maximum intensity and frequency, no great difference occurred with annealing temperature. The hardness was directly proportional to both the intensity and the frequency of the blow.

M45. A Comparative Study of Silicone Impression Materials.—*S. Rubinstein, C. W. Fairhurst, and G. Ryge, Department of Dental Materials, Marquette University, Milwaukee, Wisconsin.* Seven brands of silicone impression materials were tested in accordance with the specification testing methods proposed by Miller, Hansen, Dickson, and Sweeney of the Dental Materials Section at the National Bureau of Standards. The materials complied with the specification with respect to 12 per cent deformation, deterioration, reproducibility, and compatibility, and working time. Most of the specimens did not meet the specification standards on strain in compression, and none of them fell within the dimensional change limit of 0.2 per cent after 6 hours from mix. The proposition was made that certain portions of the specification, as they apply to silicone impression materials, be considered for modification. Additional data were compiled on flow, stiffness, and certain variations in testing the dimensional stability. The flow was large enough after a period of 24 hours to have considerable influence upon the accuracy of the impression, but it was of little significance during the first few hours after mix. The stiffness of the materials was tested with a Shore Durometer and showed a relationship of indirect proportionality with the data on strain in compression. A perforated tray seemed to have retentive power in a buccolingual direction, but not mesiodistally.

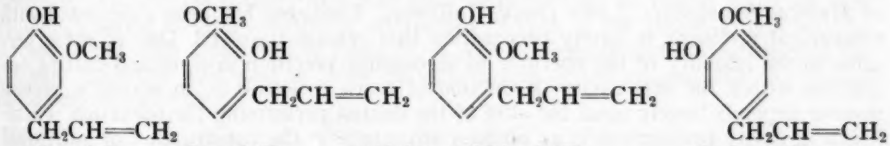
M46. Physical Properties of Elastic Duplicating Materials.—*R. G. Craig and F. A. Peyton, University of Michigan, Ann Arbor.* The stress-strain properties, compressive strengths, tear strengths, per cent set, crushing times, gelation temperatures, and duplicating qualities of six duplicating compounds were determined and compared with properties of three agar impression compounds. In addition, the properties of a number of experimental agar gels were examined. The complete stress-strain curves were obtained in less than 3 seconds and, as a result, closely reflected the properties of these materials as used in practice. The strength values were found to be a function of agar concentration and the type of duplication compound (agar or plastic). The compressive and tear strengths of agar impression or duplicating compounds were shown to be a function of deformation rate, the values reaching a maximum at approximately 10 inches/minute. The per cent set test was found to be rather insensitive to minor variations in agar materials. It was also observed that the recovery characteristics of duplicating compounds should be studied in combination with per cent set values in order to obtain a correct evaluation. The time required to rupture a material at a constant stress, or crushing time, was found to be the most sensitive test for evaluating small differences in agar materials, possibly because it measures both elastic and viscoelastic characteristics.

M47. Some Properties of Laboratory Duplicating Materials.—*Bernard Duke and Gunnar Ryge, Marquette University, Milwaukee, Wisconsin.* At the present time a specification for laboratory duplicating materials does not exist. In order to evaluate these products, five brands of duplicating material were tested for compliance with American Dental Association Specification No. 11. In addition, testing was conducted after extended storage and repeated heating-gelation cycles. Tests were also carried out for reproduction of detail and compatibility with a gypsum model. Five brands of hydrocolloidal impression materials were tested in a similar manner for comparison. It was found that the laboratory duplicating materials gave acceptable reproduction and compatibility but did not meet the specification requirement for compressive strength. The materials either did not meet the specification requirements for strain in compression and permanent set or were near the upper limit for these properties. The difference between duplicating and impression materials in their compliance with the

specification became more pronounced as the number of boilings and the storage time were increased. It is concluded that the present specification for hydrocolloidal impression material could be modified to include requirements for laboratory duplicating materials of the hydrocolloidal type.

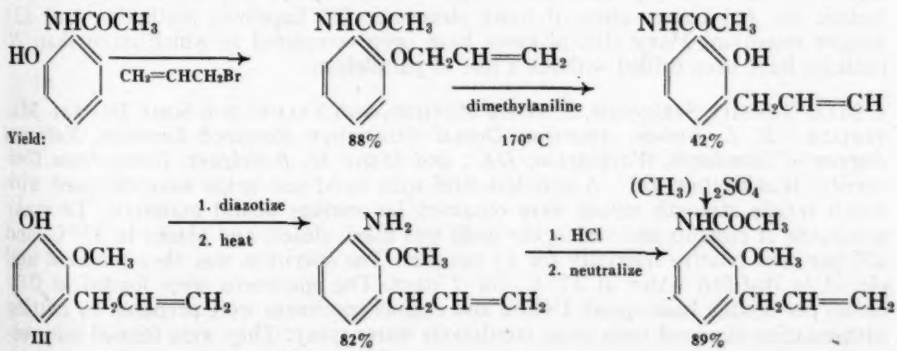
M48. ACCURACY IN PARTIAL-DENTURE-MODEL DUPLICATION.—*Richard G. Klug and Gunnar Ryge, Marquette University, Milwaukee, Wisconsin.* Various procedures influencing the accuracy of duplicated stone models were studied by means of the contour meter. Measuring points were established at several locations in master models, and a system was devised by which the location of each of these points could be defined and recorded in a three-dimensional co-ordinate system. Duplicates of the master models were fabricated (*a*) after one to twelve heating and gelation cycles, (*b*) after extended storage of the duplicating material above its gelation temperature, and (*c*) after storage of the negative in the duplicating flask, in 100 per cent relative humidity for 1, 3, 6, and 24 hours, or in K_2SO_4 solution for periods of 2, 15, 30 seconds, 1, 2, and 10 minutes, and 1, 2, 3, 6, and 24 hours. The position of the measuring points on the duplicate models was measured by the contour meter, and the changes in the three co-ordinates were tabulated. It was found that exposure to K_2SO_4 solution for 2 minutes was sufficient to produce excellent stone surfaces, and greatest accuracy was obtained when models were poured shortly after this treatment. Pouring the subsequent models in the same negative resulted in distortion and loss of surface detail. Dimensional changes of the order of 1 per cent were measured after twelve heating and gelation cycles, and after 24 hours' storage in 100 per cent relative humidity or in K_2SO_4 solution.

M49. SYNTHESES OF EUGENOL ISOMERS.—*Gerhard M. Brauer and Willard B. Howe, National Bureau of Standards, Washington, D.C.* Three-position isomers of eugenol are capable of forming chelates with metal oxides:



Eugenol (I) o-Eugenol (II) 2-Methoxy-3-allylphenol (III) Chavibetol (IV)

These compounds were synthesized to study their reactivity with zinc oxide and to determine the effect of neighboring groups on the setting mechanism. *o*-Eugenol (II) was prepared in good yield by the procedure of Allen and Gates. Possibly because of the steric effect of the neighboring allyl group, the material hardened only slowly. Two methods for the synthesis of 2-methoxy-3-allylphenol were investigated. The most suitable procedure starting with *o*-acetamidophenol is as follows:



The third isomer, chavibetol (2-methoxy-5-allylphenol, IV) was obtained by reacting methyl magnesium iodide with methyleugenol as reported in the literature. However, separation of the resulting eugenol-chavibetol mixture proved cumbersome. Other synthetic routes studied for preparation of these compounds, their characterization and physical properties, as well as their reaction with metal oxides, will be reported.

M50. Physical Properties of the Zinc Oxide–Eugenol Impression Pastes.—*G. E. Myers, University of Michigan, Ann Arbor.* Some physical and clinical properties of fifteen zinc oxide–eugenol impression products were investigated. The initial and final set times, consistency, hardness, and flow qualities of the set impression, separation from a cast, and the reproduction of detail were determined. Initial set times varied from 1.5 to 4.0 minutes, and nine of the products had values of less than 3.0 minutes. Final set times as determined with a ¼-pound Gillmore needle ranged from 3.0 to 15.0 minutes, at room temperature and humidity. Using a modified slump test for consistency, average disk sizes for the fifteen products varied from 27 to 50 mm. Products with a value greater than 37 mm. can be described as free-flowing in clinical use; those with disk sizes less than 37 mm. were of a buttery consistency. The hardness of the set impression, ½ hour after the beginning of the mix, was determined for seven products by means of a Krebs penetrometer and an ASTM D-5 needle with a total load of 100 gm. Three soft-set products had values of 1.5 mm. or greater. Four hard-set products had values of 1.0 mm. or less. The percentage flow of cylindrical specimens of eight products under a load of 1 kg. was measured at 5 minutes, 1 hour, and 24 hours after final set. Values at 5 minutes after mix ranged from 4 to 47 per cent. All fifteen products separated satisfactorily from a stone cast and were able to reproduce the finest line, 0.0015 inch, on a test block.

M51. Technics for Utilizing Intraoral Aligning Devices for Achieving Parallelism in Cavity Preparations.—*Lloyd Baum, School of Dentistry, College of Medical Evangelists, Loma Linda, California.* Dentistry has been confronted with mechanical problems in cavity preparation that remain unsolved. One of these pertains to the inability of the operator to accomplish precision drilling and cutting operations within the oral cavity. Reduction of tooth structure in an accurate, precise manner depends largely upon the skill of the dentist performing the operation. Parallelism in cavity preparation is an obvious advantage in the construction of individual cast-gold restorations, in fixed partial-denture prosthesis, and in splints for periodontal therapy. Because of limited access and visibility in the oral cavity, the use of intraoral aligning devices is not popular in the practice of dentistry in America today. This is a report of results obtained from experimentation in the development of mechanical aligning devices coupled with clinical applications of their use. With the use of special spirec drills and paralleling devices, multiple miniature pinholes may be placed quickly and easily. Associated technics permit the fabrication of dental castings to fit these cavity preparations. Clinical benefits which may be derived from this technic are (1) conservation of tooth structure, (2) improved aesthetics, and (3) greater retention. Many clinical cases have been completed in which more than 20 pinholes have been drilled without a loss of parallelism.

M52. Tensile Strength, Testing Method, and Values for Some Dental Materials.—*R. L. Bowen, American Dental Association Research Division, National Bureau of Standards, Washington, D.C., and Mario M. Rodriguez, Georgetown University, Washington, D.C.* A stainless-steel split mold and grips were designed with which tensile strength values were obtained for various dental materials. To make specimens of cements and resins, the mold was filled, closed, and placed in 37° C. and 100 per cent relative humidity for 15 minutes. The specimen was then removed and placed in distilled water at 37° C. for 7 days. The specimens were loaded at 0.02 inches per minute head speed. Dentin and enamel specimens were prepared by cutting with rotating diamond tools using continuous water spray. They were formed into rec-

tangular cross-section rods with a narrowed middle isthmus portion and notched ends. The middle portion was wound with a strip of heavy tinfoil until it fitted snugly into the stainless-steel mold. The notched ends were imbedded in direct filling resin. After the resin had hardened, the flash was trimmed and the foil strip unwound. Results obtained are given in the table.

Material	Brands	Total Specimens	Average Tensile Strength (psi)	Standard Deviation
Zinc phosphate	1	5	350	75
Silicate cements	4	20	500–1,000	100–300
Direct filling resins	2	10	4,000–5,000	500–600
Reinforced acrylic resin*	.....	12	4,000	600
Silica-resin material†	.....	9	5,000	500
Bovine dentin	.....	8	8,500	1,600
Human dentin	.....	8	5,800	900
Bovine enamel	.....	8	3,000	800
Human enamel	.....	5	1,200	350

* Methyl methacrylate resin combined with vinyl silane–treated clear fused silica.

† About 70 per cent vinyl silane–treated clear fused silica combined with about 30 per cent of an adduct of glycidyl methacrylate and bisphenol A.

M53. SOME RHEOLOGIC ASPECTS OF DENTAL MATERIALS.—*Richard H. Roydhouse, Northwestern University, Chicago.* A review of dental and other literature since 1900 reveals few reports upon the rheologic properties of dental materials. The importance of understanding and controlling such properties was discussed in relation to clinical dentistry, and, after some rheologic considerations, possible minimum criteria were suggested. Various consistency and flow measurements used on dental materials were examined, and a suitable measuring instrument in view of the above criteria was described, along with a technic for analysis. A series of experiments using alginate impression materials was used to show the application of the criteria and the clinical usefulness of rheology.

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Proceedings of the Thirty-eighth General Meeting Chicago, Illinois, March 17, 18, 19, 20, 1960

The thirty-eighth meeting of the I.A.D.R. opened with a session of the Council at 10:30 A.M., Thursday, March 17. On each following day the Council met from 12:00 noon until just before the afternoon scientific sessions.

COUNCIL ACTIONS

More than two-thirds of the councillors were present at each meeting.

The committee on the selection of an Executive Secretary recommended that the Association designate the A.I.B.S. (American Institute of Biological Sciences) as business office of the Association, with one of the officers of the A.I.B.S. to be appointed Assistant Secretary-Treasurer of the I.A.D.R. as provided in the constitution. After discussion the Council unanimously approved the committee's recommendation and directed that the matter be brought before the general meeting of the Association. Mr. F. C. Harwood of the A.I.B.S. attended one of the Council sessions to discuss the matter and to answer questions.

Annual dues for 1961 were set as follows: North American Division members, $15.00 ($7.00 for subscription to the *Journal of Dental Research* and $8.00 for the general fund); members outside North America, $3.00 dues plus $7.00 for the subscription to *JDR* if desired.

The Council directed that plans be made for the 1961 general meeting along the following lines: Prepared forms for abstracts will be sent out by the Secretary or Assistant Secretary. Abstracts must be submitted on the prepared forms, from which the abstracts will be taken without editing or recopying for production of the Preprinted Abstracts. Abstracts not on the prepared forms or not in correct form or too long will be returned to the author. The size and organization of the program are to be determined by the Program Committee on the basis of the facilities available and the papers submitted. The deadline for receipt of abstracts will be Thursday, December 1, 1960.

The Council strongly favored changing the time of the annual meeting to late June instead of March because of weather difficulties in March.

Editor Orland reported on behalf of the publication committee and submitted a list of names for the editorial advisory board as follows: Gerrit Bevelander, Gerald J. Cox, Alton K. Fisher, Joseph Gibilisco, Stanley C. Harris, T. Ockerse, Floyd D. Ostrander, Gunnar Ryge, John J. Salley, Robert M. Stephan, Donald A. Wallace.

Nominations for election to membership presented by the Membership Committee were approved by the Council. Because of the large number of names on the list, the Council directed that the list be posted for inspection prior to the final general meeting of the Association. This was done.

Barnet M. Levy and William T. Sweeney were elected members of the *ad interim* committee.

John R. Ring, chairman of the Membership Committee, reported that the committee recommends a change in the procedures for nomination and election of members. The committee was directed to present a petition for amendment to the constitution to accomplish the change. The petition was presented and approved by the Council for submission.

Ellis B. Jump reported for the Edward H. Hatton Award Committee, recommending that eligibility for the awards should be redefined. Members who have ideas on the subject should communicate with their councillors.

Ned B. Williams reported for the Past Presidents to the effect that funds should be sought to establish a foundation for research in oral health. A committee to study ways and means was appointed as follows: J. F. Volker, Chairman, Maynard K. Hine, Ned B. Williams, H. T. Knighton, J. A. English, and S. J. Kreshover.

To expedite the process of nominating candidates for election as officers of the Association, the Nominating Committee of the Council met and presented their selections for the 1961 election. Council approved the selections as Council nominees for the 1961 election as follows: President-elect, S. J. Kreshover; Vice-President, Dan Y. Burrill; Secretary-Treasurer, Joseph C. Muhler; Publication Committeeman, Richard S. Manly; Councillors-at-large, Josse E. deWever and Clifton O. Dummett.

EXECUTIVE SESSION I, MARCH 17, 1960, 8:00 P.M.

More than a quorum was present. The minutes of the 1959 meeting, as printed in the proceedings of the meeting in the *Journal of Dental Research,* August, 1959, Vol. **38,** No. 4, pp. 767–75, were approved.

Erling Johansen, Josse E. deWever, and Benjamin W. Warner were appointed tellers to count the ballots for election of officers.

The financial report of the Secretary-Treasurer was presented and approved. The report is reproduced in the supplement (C and D).

Approval was obtained for expenditures of Association funds (previously approved by Council) as follows: (1) for payment of all expenses of this meeting; (2) expenditure of excess funds of the Association for the support of the *Journal,* if needed, on approval of the *ad interim* committee; (3) a president's fund of not more than $125 to be used by the president in entertainment on behalf of the Association at this meeting.

A report by Editor Orland on behalf of the Publication Committee was presented and approved (see suppl., B).

Bernard K. Forscher reported on the XXI International Congress on Physiology held in August, 1959, in Buenos Aires. Dr. Forscher, Dr. Leon Kraintz, and Dr. Frederick M. Lieban were assisted in attendance at the congress by a grant to the Association from the National Institutes of Health.

Ned B. Williams reported on the 1959 International Conference on Oral Biology held in New York in September, 1959. This conference, the first of its kind, was made possible by a grant to the Association from the Colgate-Palmolive Company. The conference was well attended and judged very successful. President Robinson announced the receipt of a $30,000 grant from the Colgate-Palmolive Company for another International Congress on Oral Biology to be convened in Europe in 1962. A committee was appointed to plan the conference, as follows: Ned B. Williams, H. T. Knighton, J. A. English, S. J. Kreshover, J. E. deWever, Hamilton B. G. Robinson, and representatives from other countries to be announced. The committee was instructed to select its own chairman and to start planning at this meeting.

Secretary-Treasurer Burrill, chairman of the committee appointed to select an executive secretary, reported the suggested arrangement with the American Institute of Biological Sciences, that was previously approved by the Council. The A.I.B.S. is to take over the routine business of the Secretary-Treasurer's office at a cost not to exceed $2.00 per member. The Secretary-Treasurer urged that this arrangement be approved. The burden of routine business becomes greater as the Association grows, and it is felt, furthermore, that the Association should not continue to have a quasi-permanent elected officer. After extended discussion, the arrangement with the A.I.B.S. was approved, expenditure of Association funds for the purpose was approved, and the Secretary-Treasurer was directed to proceed in establishing the A.I.B.S. as Executive Secretary and business office for the Association. Howard F. Campbell, financial officer of the A.I.B.S., was designated Assistant Secretary-Treasurer of the I.A.D.R.

The Secretary-Treasurer proposed an amendment to Section C, 2 (A) of the bylaws whereby the Vice-President would be a member of the Program Committee in place of the Secretary-Treasurer, who would be a member ex officio. This amendment was adopted.

The President proposed an amendment to Section F 3, (C) of the bylaws for the purpose of eliminating a difficulty in the provision for a nominating committee. The amendment requiring a nominating committee of Associaton members rather than Council members was adopted.

The meeting adjourned to 5:00 P.M., Sunday, March 20, 1960.

Immediately after the adjournment the Directors of the I.A.D.R., a corporation not for profit under the laws of the state of Illinois, met and approved the conversion of old bonds in the endowment fund of the Association to new government securities as advised by the trust officers of the approved bank.

EXECUTIVE SESSION II, MARCH 20, 1960, 5:00 P.M.

More than a quorum was present. The meeting convened as a session of the North American Division of the I.A.D.R. Joseph F. Volker and James A. English were elected representatives of the I.A.D.R. to the council of the A.A.A.S. D. Walter Cohen was elected representative of the I.A.D.R. to Section Nd of the A.A.A.S. Reidar F. Sognnaes urged members to apply for fellowship status in the A.A.A.S. There being no further business, the meeting adjourned and immediately reconvened as a general executive session of the I.A.D.R.

On motion duly voted, the thanks of the Association were expressed to the members of the Local Arrangements Committee under the chairmanship of Frank M. Wentz and to Max Shapiro, chairman of the Public Relations Committee, for their contributions to the success of this meeting.

On recommendation of the Council, one hundred and fifty-six new members were elected unanimously (see suppl., H, for names). Dr. George White Whipple was nominated by the Council for election as an honorary member of the Association. Election was unanimous.

The Secretary-Treasurer requested and received approval of a budget for his office (see suppl., E). It was stated that the costs of transfer of routine business to the A.I.B.S. were not known but would probably not exceed the budget.

The State Bank and Trust Company of Evanston, Illinois, and the Riggs National Bank of Washington, D.C., were approved as repositories of Association funds.

Expenditures of funds in the *Journal* account for expenses of the *Journal* were approved.

The President reported a council recommendation that the Program Committee and the Public Relations Committee be authorized to spend up to $1,000 for public relations assistance for the 1961 meeting. Approval and authorization of the expenditure were voted.

The Association voted approval of a Council recommendation that the Program Committee be authorized to meet to organize the program for the 1961 meeting and that costs of such meeting be borne by the Association.

A recommendation from the Past Presidents that the Association should investigate ways of obtaining funds to create a foundation for the support of dental research was presented. The committee recommended by the Council was appointed.

The Committee on Meeting Places reported the following locations: 1961, Boston; 1962, St Louis; 1963, Pittsburgh; 1964, Los Angeles; 1965, Toronto; 1966, Kansas City or Chicago.

John R. Ring presented a petition for amendment of the constitution, duly signed by five members, allowing for change in the procedure of election to membership. The amendment will be on a ballot sent to all members and will be voted on at the 1961 meeting. The amendment is reproduced in the supplement (G).

The President reported the recommendation of Council that the Association seek and accept grants to establish additional or expanded awards for meritorious research in dentistry.

On motion unanimously voted, the Association expressed its thanks for grants received from the Colgate-Palmolive Company, the National Institutes of Health, the Gies Foundation, and the many supporting Associates of the *Journal*.

Joseph F. Volker presented a resolution on the passing of Dean Milton Winternitz (suppl., I). The Secretary-Treasurer read the names of members known to have died during the year, including the venerable Edward H. Hatton, for years I.A.D.R. Secretary-Treasurer. The meeting stood silent in respect.

The President expressed his appreciation to the officers and committees for their work during the past year.

The report of the tellers was received, and the officers for the coming year were declared elected and were duly installed. Newly elected President Knighton took the chair and recognized Dr. Joseph Muhler. The latter commended Past President Robinson for his efforts and efficiency during his year as president and called for a standing vote of thanks.

The meeting adjourned sine die.

SUPPLEMENT TO PROCEEDINGS

ATTENDANCE AT COUNCIL MEETINGS

The following were present at one or more of the four meetings: S. S. Arnim, G. Bevelander, B. R. Bhussry, P. E. Boyle, D. Y. Burrill, Mary C. Crowley, E. C. Dobbs, J. A. English, A. K. Fisher, L. S. Fosdick, J. Gibilisco, R. L. Glass, P. Goldhaber, G. E. Green, W. C. Hess, E. Johansen, H. T. Knighton, R. S. Manly, J. C. Muhler, F. J. Orland, G. C. Paffenbarger, J. R. Ring, H. B. G. Robinson, Genevieve D. Roth, D. E. Shay, E. L. Smith, W. T. Sweeney, P. R. Weisenstein, N. B. Williams, J. Wisotzky.

B. REPORT OF THE EDITOR

In its forty-one years, the *Journal of Dental Research* has had a face-lifting performed to a lesser or greater degree on six different occasions. These changes in format, I thought, could best be illustrated by means of a simple exhibit. This we prepared at no cost to the *Journal* account; it is purely a home-based activity on the part of several interested persons. The theme of this little exhibit points out the extensive growth of the *Journal* over the many years of its existence. There has been published, as a grand total, 2,381 manuscripts, 4,087 abstracts, and 32 short research notes upon a total of 27,558 pages since the beginning in March, 1919.

Needless to emphasize, a huge proportion—almost half—of this publishing activity occurred in

the last decade, during our unique period of economic and research prosperity. In the exhibit there is also illustrated on a map the fact that manuscripts published during the past year have come from 13 different countries and 53 different cities or centers of dental research. As in other years, the United States predominates as the major site of such research, at least as determined by published reports in the *JDR*. Subscribers come from many different lands (actually, 39), but the vast majority of reader-subscribers live in the United States. Over the last four decades there has been a shift in topics covered by manuscripts. Electron microscopy and fluorides were unheard-of subjects in 1919, while in 1959 there were, respectively, 16 and 27 papers published in these fields of endeavor.

In the past year (1959), Volume **38** consisted of some 1,264 pages of published material. One hundred and twenty-nine manuscripts were published, and, of these, 101 were revised by the authors. Thirty-four papers were rejected, but some of these were accepted as short research notes. There were 348 abstracts and 26 research annotations published during 1959.

The processing time from the day a manuscript is received and acknowledged by the editorial office until the appropriately printed *Journal* is in the mail has run about 7–8 months, on the average. This is a little longer than last year. It is about 3–4 months until the *Journal* is out from the time an author's revision is received, on the average. It is our editorial hope that this time lag

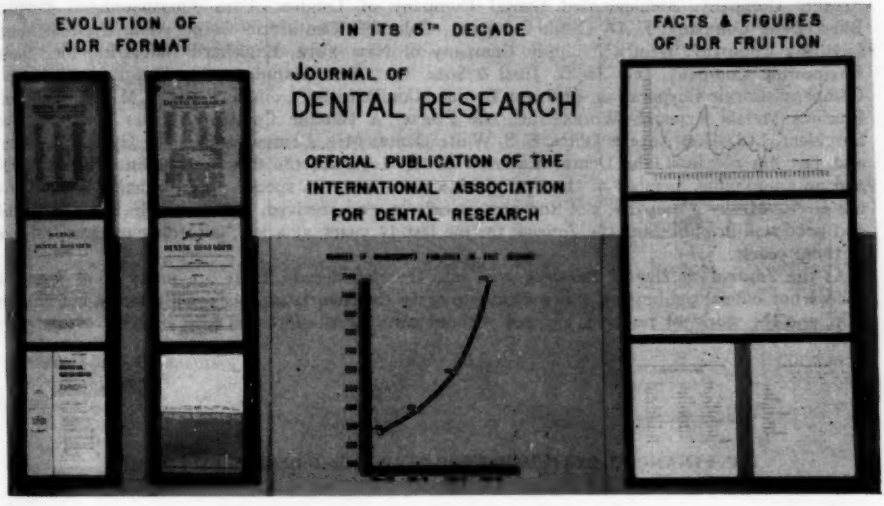

FIG. 1.—*Journal of Dental Research* Exhibit

can be further reduced, but there are several problems to be surmounted in this area. Yet the short notes—research annotations—have appeared in the *Journal* usually in the next or almost next number going to press.

The first issue of 1960 in its appearance is different. The front cover follows the trend of simplicity seen in certain other journals. The style of type, journal size, and arrangement somewhat follow the pattern of other University of Chicago journals. By action last year of the Editor, the Publication Committee, and the Council, the University of Chicago Press has become the printer of the *Journal*. The University Press is an educational, non-profit institution operating its own printing plant, where the *Journal* is processed and printed, across the street from the editorial office of the *Journal*.

The editor wishes to thank the many members for their congratulatory letters and comments on the new look of the *Journal*. As with any item being produced in its first run, there are problems and wrinkles to be ironed out. I also wish to thank those of you who have taken the time to scrutinize the recent issue and inform me of needed improvements. The University Press itself, as well as the editorial office, early pointed up some of the shortcomings. These problems will be improved upon in subsequent issues.

Under present arrangements, where the Editor and assistants are in closer touch with the *Journal* processing by the Press, we have been impressed with how much additional work must go into publishing a manuscript when it is not adequately prepared by the author. Illustrations should be

of the highest quality, including the lettering and symbols, or they will not reproduce well. We are going to raise our standards of acceptance in this area in the coming year. Many papers received in the past year were commendable, with minor revisions required in most cases. Most authors have expressed appreciation for the review comments of their papers. There are still delays in reviewing, editing, and processing which will be improved upon, whenever and wherever possible, but our mail, both coming and going, is still very heavy.

At this point I might mention that, as a time-honored custom, established by Hamilton B. G. Robinson, my predecessor, the *Journal* will accept revisions of abstracts during the time of the meeting.

At this time I should like to commend the several members who have completed a full 2-year term on the Advisory Editorial Board: Finn Brudevold, Harold C. Hodge, Seymour J. Kreshover, Barnet M. Levy, Richard S. Manly, Sholom Pearlman, Ralph W. Phillips, Jens J. Pindborg, Leon H. Schneyer, David B. Scott, William G. Shafer.

No matter who prints or publishes the *Journal*, publishing and printing costs are high these days, and any monetary help that the *Journal* and its Publication Committee received is deeply appreciated. We thank the following groups for monies currently received for the 1960 year: the William J. Gies Foundation, the United States Public Health Service, the Colgate-Palmolive Company, the Acralite Company, Amalgamated Dental Company of London, Astra Pharmaceutical Products, Block Drug Company, L. D. Caulk Company, Columbia Dentoform Corporation, Crescent Manufacturing Company, Dentist's Supply Company of New York, Englehard Industries, Inc., Hanau Engineering Company, Inc., H. D. Justi & Sons, Inc., Kerr Manufacturing Company, Eli Lilly & Company, Lorvic Corporation, Midwest Dental Manufacturing Company, the J. M. Ney Company, Precious Metals Research Works, Inc., the Procter & Gamble Company, Ritter Company, Inc., Surgident, Ltd.—Los Angeles Office, S. S. White Dental Mfg. Company, Inc., the Upjohn Company, and, last but not least, the Dental Materials Group I.A.D.R., the Chicago section of the I.A.D.R., and an anonymous member of the Dental Materials Group. A special note of thanks should go to the C. V. Mosby Company, not for any specific money received, but for their tremendous help and good will in publishing the *Journal* for the last 12 years, at a loss, according to them, for most of those years.

As the *Journal of Dental Research* goes into its fifth decade, there are many good reasons to believe our official publication will continue to serve the Association, its rapidly increasing membership, and the world of research, but not without continuous effort and help from all of us.

FRANK J. ORLAND
Editor

C. FINANCIAL STATEMENT FOR YEAR ENDED DECEMBER 31, 1959

Wilmette, Illinois
March 15, 1960

International Association
for Dental Research

GENTLEMEN:

The accompanying statements of the International Association for Dental Research, showing the Receipts and Disbursements of the General Fund and the Endowment Fund for the Year Ended December 31, 1959, and the Receipts and Disbursements of the Journal for Dental Research Fund for the period, March 16, 1959 to December 31, 1959, have been reviewed and the respective items traced into the related bank statements. No other verification was made of receipts from members for dues and subscriptions and no physical examination was made of the U.S. Government Bonds shown in the Endowment Fund. Such other procedures of verification have been followed as I considered necessary in the circumstances.

In my opinion, the accompanying statements fairly present the receipts and disbursements of these three Funds of the International Association for Dental Research on a cash basis for the periods indicated, consistent with the procedures followed in prior years except for the separation now made of the Endowment Fund.

Signed
DALE H. TAYLOR
Certified Public Accountant

GENERAL FUND

Receipts and Disbursements
For Year Ended December 31, 1959

Fund Balance January 1, 1959			
On deposit at State Bank and Trust Company, Evanston, Illinois		$11,089.08	
Savings Account		248.01	
Petty Cash and Receipts not deposited		26.85	
Total Fund Balance			$11,363.94
Receipts			
Subscriptions*		$ 5,721.00	
Dues†		6,094.88	
Registration Fees and Sale of Banquet Tickets, Annual Meeting		2,755.50	
Colgate-Palmolive Co. grant for travel expense to International Conference of Oral Biology		30,000.00	
Total Receipts			44,571.38
			$55,935.32
Disbursements‡			
Printing and Distributing Journal (C. V. Mosby Co.)		$ 5,842.50	
Mailing operations, postage, and printing		561.91	
Gratuity to Secretary-Treasurer		500.00	
Annual Meeting expense,			
Meeting facilities, banquets, and luncheons	$1,249.54		
Printing programs, badges, etc.	713.92		
Novice Award	100.00		
Projection and Public Address	812.13		
Other Expenses	157.96	3,033.55	
American Association of Biological Sciences		100.00	
To *Journal* Fund for publishing Abstract		1,000.00	
Office expenses			
Clerical and professional	$ 192.50		
Other	96.95	289.45	
Travel expenses, 97 delegates to International Conference of Oral Biology		24,442.60	
Expenses, International Congress of Physiology and Pharmacology		2,337.18	
Separation of Endowment Fund, beginning Balance		289.41	
Total Disbursements			$38,396.60
Fund Balance, December 31, 1959§			
On deposit at State Bank and Trust Company, Evanston, Illinois		$17,565.98	
Less, due Petty Cash		(27.26)	
Total Fund Balance			$17,538.72

ENDOWMENT FUND

Receipts and Disbursements
For Year Ended December 31, 1959

Beginning Balance transferred from General Fund accounting report for the previous year			$ 289.41
Other Receipts			
Interest from U.S. Government bonds		$ 41.40	
Interest from Savings Accounts‖		312.08	
Proceeds from redemption of U.S. Government Series E Bonds		75.00	
Total Other Receipts			428.48
			$ 717.89
Less, Purchase cost of one $100 U.S. Government Series E Bond			75.00
Balance in Savings Account, State Bank and Trust Company, Evanston, Illinois			$ 642.89

* Subscriptions received are related to various years as follows: 1958 and prior—$133.00; 1959—$4,012.00; 1960—$1,575.00 1961—$1.00.

† Dues received are related to various years as follows: 1958 and prior—$176.00; 1959—$4,187.88; 1960—$1,731.00.

‡ The accompanying statements have been prepared on a Cash Basis and do not reflect certain current obligations of the Association which had not been paid at December 31, 1959.

§ In addition to the Cash and other assets shown, the Association owns equipment which cost $577.19.

‖ The interest from Savings Accounts includes $300.00 earned from General Funds temporarily held in the Endowment Fund Savings Account. Per Dr. D. Y. Burrill, these earnings will remain in the Endowment Fund.

U.S. GOVERNMENT BONDS

Value at January 1, 1959, per prior report		$1,650.00
Add, One $25 Bond received from custody of other officers		25.00
Total cost of U.S. Government Bonds held, composed of		
Series K Bonds	$1,500.00	
Series G Bonds, now matured	100.00	
Series E Bonds, dated August, 1959, at cost (par $100)	75.00	
		$1,675.00

D. REPORT OF *Journal of Dental Research* FUNDS

Receipts and Disbursements
For the Period
March 16, 1959, to December 31, 1959

Fund Balance, March 15, 1959			
On deposit at State Bank and Trust Company, Evanston, Illinois			$22,459.41
Receipts			
Supporting Associates		$ 4,450.00	
Colgate-Palmolive Co. for student subscriptions#		19,151.00	
Transfer from General Fund toward abstract costs		1,000.00	
Sale of back issues		662.85	
Total Receipts			25,263.85
			$47,723.26
Disbursements			
C. V. Mosby Co. for extra pages		$ 5,079.00	
C. V. Mosby Co. for student subscriptions from Colgate-Palmolive Grants		17,651.00	
University of Chicago, services to *Journal of Dental Research*	$ 4,635.67		
Subsidy toward publication of 1960 *Journal*	10,000.00	14,635.67	
Shipping charges, back issues		40.90	
Advanced to D. F. Mitchell for Petty Cash expenditures		54.03	
Total Disbursements			37,460.60
Fund Balance, December 31, 1959			
On deposit at State Bank and Trust Company, Evanston, Illinois			$10,262.66

E. BUDGET FOR 1960–61

Office of Secretary-Treasurer			
Direct Expense			
Clerical	$ 250		
Postage and printing	750	(Note: The amounts in each of the categories of direct expense are approximate. Transfers from one category to another as necessary are approved.)	
Supplies	150		
Travel and misc.	50		
E. H. Hatton Award	100		
Contingency Fund	200		
Gratuity	500		
Secretarial service	1,500		
Total	$3,500.00		
Contingency fund for Editor of *JDR*			$1,000
Contribution toward cost of publishing abstracts in *JDR*			$1,000

This grant is for different years as follows: 1959—$1,651.00; 1960—$17,500.00.

F. AMENDMENTS TO THE BYLAWS UNANIMOUSLY ADOPTED AT THE MEETING, MARCH 17, 1960

Section A. Membership 1.—Nomination now reads: "Nominations for membership shall be signed by two members of the Association who shall attest to the eligibility of the nominee for membership."

Section C. Meetings 2.—Program (A) now reads: "The arrangements for each annual meeting of the Association shall be made in accordance with the instructions from the Association or the Council by a Program Committee consisting of the President-elect, the President, and the Vice-President, with the Secretary-Treasurer as a member ex officio."

Section F. Committees 3.—Standing committees (c) now reads: "A nominating committee of five members to advise the Council in the selection of members of the Association for nomination as candidates for offices on the official ballot of the Association." (The amendment deleted the word "Council" preceding "members.")

G. PETITION FOR AMENDMENT OF THE CONSTITUTION OF THE I.A.D.R. PRESENTED AT THE MEETING, MARCH 20, 1960

"WHEREAS, The membership requirements of this Association have been revised and greater numbers of nominations for election to membership are anticipated, and

WHEREAS, The business administration of the Association is about to undergo certain changes in operation, and

WHEREAS, The current method of nominating and electing members is becoming increasingly unwieldy,

Therefore the undersigned duly propose the following amendment to the Constitution of the I.A.D.R.:

Article III, Membership.—Sections 2, 3 and 4 are repealed, and in their place the following substituted:

Section 2, Election.—Any person who is eligible for election to membership under Article III, Section 1, who shall have been nominated in writing in due form by two members of the Association, and who shall have been certified by the Secretary-Treasurer or Assistant Secretary-Treasurer and approved by the membership committee shall become a member of the association a reasonable time after the notice of his nomination has been mailed to the members of the association, provided no member of the association gives notice of objection to the election within such reasonable time.

Article III.—Section 5, shall be renumbered Section 3.

Signed: J. R. RING
GEORGE C. PAFFENBARGER
DAN Y. BURRILL
H. B. G. ROBINSON
S. J. KRESHOVER

H. ELECTIONS TO MEMBERSHIP, MARCH 20, 1960

NORTH AMERICA.—Irving I. Abramson, *Balitimore, Md.;* Robert Joseph Adams, *Memphis, Tenn.;* Apostolos X. Apostolopoulos, *Rochester, N.Y.;* Howard Anthony Arden, *Scarsdale, N.Y.;* Ralph Carson Appleby, *Iowa City, Ia.;* Arthur Nathaniel Bahn, *Evanston, Ill.;* Joseph J. Barone, *Bethesda, Md.;* Charles Edward Barr, *Norfolk, Va.;* James Overton Beck, Jr., *Indianapolis, Ind.;* James H. Belding, *Davenport, Ia.;* William V. Bernard, *San Francisco, Cal.;* W. Robert Biddington, *Baltimore, Md.;* Joseph C. Biddix, *Baltimore, Md.;* Arne M. Bjorndal, *Iowa City, Ia.;* Robert V. Blackmore, *Rochester, N.Y.;* Billy Jim Blankenship, *Houston, Tex.;* Harry Blechman, *New York, N.Y.;* Theodore E. Bolden, *Jersey City, N.J.;* Louis J. Boucher, *Milwaukee, Wis.;* Richard E. Bradley, *Lincoln, Neb.;* Henry S. Brenman, *Philadelphia, Pa.;* Romaine R. Bruns, *Rochester, N.Y.;* Russell V. Brown, *Milwaukee, Wis.;* George W. Burke, Jr., *Richmond, Va.;* Leslie R. Burrows, *Rochester, N.Y.;* James William Bynum, *St. Louis Mo.;* Louis J. P. Calisti, *Westwoood, Mass.;* Joseph P. Cappuccio, *Baltimore, Md.;* Virginia M. Carbonell, *Chicago, Ill.;* Frederick M. Chacker, *Philadelphia, Pa.;* John M. Clauser, *St. Louis, Mo.;* Berson Cohen, *New York, N.Y.;* Mark H. Conner, *Bethesda, Md.;* Ruth M. Davenport, *Washington, D.C.;* Michael J. Del Balso, *Milwaukee, Wis.;* Hristo Chris Doku, *Boston, Mass.;* Frank A. Dolle, *Baltimore, Md.;* William B. Donohue, *Montreal, Canada;* Homer L. Dorman, *Dallas, Tex.;* Martin Dworkin, *Indianapolis, Ind.;* Emrich

E. Elliott, *Chicago, Ill.;* A. Bernard Eskow, *Baltimore, Md.;* Richard L. Farquhar, *Pittsburgh, Pa.;* William Marion Feagans, *Richmond, Va.;* Conrad C. Ferlita, *Baltimore, Md.;* Helmi R. Fogels, *Boston, Mass.;* William C. Foster, *Philadelphia, Pa.;* Walter L. Gabler, *Chicago, Ill.;* Louis P. Gangarosa, *Rochester, N.Y.;* W. Arthur George, *Pittsburgh, Pa.;* Sol D. Gershon, *West Englewood, N.J.;* Charles W. Gish, *Indianapolis, Ind.;* William G. Goodale, *Iowa City, Ia.;* David H. Gould, *Leonia, N.J.;* Joseph M. Gowgiel, *Chicago, Ill.;* Poul Grøn, *Boston, Mass.;* Anna T. Hampel, *Minneapolis, Minn.;* Leland C. Hendershot, *Chicago, Ill.;* Judson C. Hickey, *Columbus, O.;* E. Henry, Hinrichs, Jr., *Ruxton, Md.;* Jean Turnbaugh Hodson, *Seattle, Wash.;* Heiner Hoffman, *Jamaica, N.Y.;* Donald F. Huelke, *Ann Arbor, Mich.;* Wen-shui Shih Hwang, *Iowa City, Ia.;* Gerald P. Ivancie, *Denver, Colo.;* Henry Jeffay, *Chicago, Ill.;* Hugh D. Killmer, *Houston, Tex.;* Jules Klingsberg, *New York, N.Y.;* William L. Kydd, *Seattle, Wash.;* Herman Henry Langkamp III, *Pittsburgh, Pa.;* Frederick M. Liebman, *New York, N. Y.;* Sangiem Limbasuta, *Rochester, N.Y.;* Martin Lunin, *Houston, Tex.;* Jean-Paul Lussier, *Montreal, Canada;* Lilia McMillan, *Chicago, Ill.;* Parker Eugene Mahan, *Rochester, N.Y.;* Alexander L. Martone, *Norfolk, Va.;* Amarjit Singh Marwah, *Chicago, Ill.;* James L. Matthews, *Dallas, Tex.;* Maurice A. Mazzarella, *Great Lakes, Ill.;* Sterling Garrett Mead, *Washington, D.C.;* José E. Medina, *Baltimore, Md.;* Valy Menkin, *Kansas City, Mo.;* Barry G. Miller, *Charlotte, N.C.;* John R. Mink, *Indianapolis, Ind.;* Fircze D. Mirza, *Chicago, Ill.;* Bernard S. Moskow, *Brookline, Mass.;* Richard W. Moss, *Wood, Wis.;* Julius Ozick, *New York, N.Y.;* Frederick A. Pflughoeft, *Wauwatosa, Wis.;* Grant T. Phipps, *Pittsburgh, Pa.;* Anthony Picozzi, *Edgewater, N.J.;* Kendall Porter, *Kansas City, Mo.;* Leonard Rapoport, *Baltimore, Md.;* Wayne L. Reeve, *Kansas City, Mo.;* Edward J. Reith, *New York, N.Y.;* Robert L. Richardson, *Iowa City, Ia.;* Marvin M. Rosenberg, *New York, N.Y.;* Samuel R. Rossman, *Philadelphia, Pa.;* Richard H. Roydhouse, *Chicago, Ill.;* Joseph Rubinstein, *Highland Park, Ill.;* Douglas J. Sanders, *Baltimore, Md.;* W. E. Sanders, *Kansas City, Mo.;* Henry C. Sandler, *Brooklyn, N.Y.;* Christian Schwabe, *Iowa City, Ia.;* Vincent A. Segreto, *San Antonio, Tex.;* Joseph H. Seipp, Jr., *Baltimore, Md.;* Haritun A. Shabrik, *Boston, Mass.;* Ira L. Shannon, *Randolph AFB, Tex.;* A. Harold Sills, Jr., *Houston, Tex.;* Jules M. Snitzer, *St. Louis, Mo.;* Narendar Nath Soni, *Indianapolis, Ind.;* John Monroe Spence, *Chicago, Ill.;* Daniel J. Spinella, *Bethel, Conn.;* Richard Elgin Stallard, *Edina, Minn.;* Irving B. Stern, *Seattle, Wash.;* Walter C. Stout, *Dallas, Tex.;* William H. Tade, *Iowa City, Ia.;* Richard G. Taylor, *Boston, Mass.;* Benjamin Vargas, *Santurce, Puerto Rico;* Martin J. Wagner, *Dallas, Tex.;* John W. Wakely, *Minneapolis, Minn.;* A. W. Ward, *San Francisco, Calif.;* Thomas B. Weber, *San Antonio, Tex.;* Sidney Weiss, *Levittown, Pa.;* Jack E. Wells, *Kansas City, Mo.;* Albert P. Westfall, *Houston, Tex.;* Charles John Wilson, *Milwaukee, Wis.;* George J. Witkin, *New York, N.Y.;* Joseph A. Yacovone, *Pawtucket, R.I.;* Seymour H. Yale, *Chicago, Ill.;* Bernard J. Zeldow, *Seattle, Wash.*

GREAT BRITAIN.—John Highet Allan, William George Cross, John Desmond Eccles, Arnold S. T. Franks, William Holgate, P. M. C. James, William Dennis McHugh, Guy Alistair Morrant, Derrick James Neill, George Sutherland Nixon, J. W. Poole, Dennis Clifford Smith, Ronald L. Speirs, Roy Storer, Arnold Richard Ten Cate.

AUSTRALIA.—D. A. Cameron, Garry Charlton, Kenneth W. Knox, Geoffrey S. Molyneux, Ernest Newbrun, Harold R. Sullivan.

OTHER COUNTRIES.—Ayoub Amer, *Cairo, Egypt;* Jun Ishikawa, *Tokyo, Japan;* Luigi Marziani, *Rome, Italy.*

I. MISCELLANEOUS

1. *Statistics:*

Current Membership	1,121	Attendance at 1960 Meeting	
North America	917	Members	381
Outside N.A.	204	Non-members	346
		Guests	93
		Total	820

2. *Officers elected for 1960–61:*

President	Holmes T. Knighton
President-Elect	James A. English
Vice-President	Seymour J. Kreshover
Secretary-Treasurer	Dan Y. Burrill
Editor (Two-year term)	Frank J. Orland
Publication Committeeman (two year term)	David F. Mitchell
Councillors-at-large	Clifton O. Dummett, Ralph L. Ireland

*Formation of Periodontal Tissues around Subcutaneously Transplanted Hamster Molars**

RICHARD L. HOFFMAN†

University of Illinois College of Dentistry, Chicago

The purpose of the present investigation is to determine whether the developing tooth has a formative influence upon the tissues which surround it and, if so, how far the influence extends and how long it lasts. In vitro microdissection studies have indicated that the enamel organ of the developing tooth exerts a variety of influences inwardly upon the mesenchyme within it—the dental papilla. Its epithelium is believed to influence the initial condensation of mesenchyme to form the dental papilla, the differentiation of odontoblasts, and the general shaping of the tooth crown and roots. However, the possibility that the enamel organ might also exert influence outwardly upon the surrounding mesenchyme of the dental sac has not been experimentally tested. The transformation of the dental sac into the definitive periodontium, consisting of the periodontal ligament and alveolar bone, proceeds simultaneously with the growth and development of the roots. This suggests some kind of co-ordinating influence between periodontium development and the development of the tooth proper. In order to test whether such an influence exists, the enamel organ and dental papilla of the developing hamster molar were dissected from their dental sac before root formation, transplanted into connective tissue which ordinarily does not form periodontium, and allowed to develop. It was found that all healthy specimens recovered after 4 weeks in the host had organized periodontium around themselves; this suggests the existence of an organizing influence emanating from the developing root. The extent of the influence was found to be limited to the periodontal ligament and a relatively thin shell of alveolar bone around the roots and bone in the space between the roots.

Cells forming bone around transplants seem to have been supplied by the host. The stimulus for conversion of host cells into osteoblasts probably came from the enamel organ and its derivative—Hertwig's epithelial root sheath. This stimulus was found to be capable of influencing the development of a periodontal ligament resembling markedly the periodontal ligament which normally develops in the jaw in the presence of both prefunctional and functional formative stimuli. Because transplants developed in the absence of normal function, differences between the periodontal ligament which developed in situ and the ligaments in transplants could then be attributed to the influence of function upon structure.

Part of a thesis submitted to the Graduate College, University of Illinois, in partial fulfilment of requirements for the degree of Master of Science.

Received for publication August 28, 1959.

* Edward H. Hatton Award winner, 37th General Meeting, 1959.

† Postdoctoral fellow, U.S. Public Health Service.

Other investigators have observed bone and/or periodontal ligament around teeth transplanted into connective tissues other than the jaw. However, these writers were concerned primarily with other aspects of tooth transplantation, and, in each case, observations of elements of the periodontium were serendipitous. Willis,[1] while testing the rat brain as a possible site for growth and development of minced embryonic rat parts, found some fully developed and some partly developed teeth in the recovered transplants of minced jaw. One fully formed tooth had bone adjacent to it, but the possibility that the bone had been transplanted with the developing tooth was not ruled out. Kostecka[2] homoplastically transplanted embryonic dog tooth germs subcutaneously and intermuscularly into young dogs. However, it is not clear why he evaluated occasional cartilage and bone formation as signs of absorption. While investigating histologic changes in tooth germs transplanted into the tibia marrow cavities of kittens, Sutro and Pomerantz[3] found bone around the roots of some specimens, as well as pulp tissue which had been transformed into bone in others. Villafane and Brero[4] autotransplanted rabbit incisors without the dental sac intermuscularly and after a year recovered the transplant and reported an encapsulated specimen which had bone on some parts of the enamel surface. Fleming[5] refers to the osteogenic activity of cells transplanted with the tooth germ into the anterior chamber of the eye. Hammer[6] transplanted fully developed teeth to the greater omentum in dogs and monkeys, where he found the preserved part of the periodontal ligament connected to the omentum by means of scar tissue. While Bejdl and Weizenberg[7] were showing that heteroplastic transplantation of tooth germs was possible among dogs, pigs, and rats, they also noticed and reported both periodontal ligament and adjacent bone around the roots of transplanted teeth.

Experiments reported in the literature indicate that the developing tooth can survive transplantation and continue growth and development in vivo and, to a limited extent, in vitro. However, as far as can be ascertained, no one has studied systematically the formation of periodontal tissues around teeth transplanted into sites other than the jaw. Transplantation into a connective tissue other than the jaw is to be distinguished from replantation in the alveolus, where healing alone may be responsible for regeneration of the periodontal ligament and where function may also contribute to the alignment of its fibers.

MATERIALS AND METHODS

Developing molars were transplanted from newborn hamsters (*Cricetus auratus*) into the subcutaneous tissues of young adult hamsters. Although both younger and older tooth germs were also transferred, only the procedure used for the neonatal transplantations will be described, since the technic is essentially the same for all ages.

Every effort was made to carry out transplantations aseptically. Sharp instruments were sterilized in zephiran chloride; all other instruments, as well as gauzes and towels used to dress the operating table and to protect instruments, were autoclaved, and host skins were shaved and swabbed with merthiolate before incisions.

Newborn developing teeth were easily seen with the aid of binocular loops. Upper first molars were transplanted in all cases. These teeth are unerupted and easily injured during removal; anlagen known to have been torn or pierced by instruments were discarded.

Neonatal donors were first decapitated. The maxilla and top of the head were then separated from the remainder of the head by inserting scissor points into the corners of the mouth on each side and cutting back through the base of the skull. With the maxilla facing upward, the upper head was placed on a glass slide and the elevated and rounded area of the developing first molar located. An iridectomy knife was inserted, from the buccal (lateral), just beneath the surface tissue of the rounded molar area, and the thin superficial tissue over the developing tooth was completely removed. Vertical incisions were made at the anterior, posterior, and buccal (lateral) edges of the tooth to sever adhesions and attachments to the second molar. By inserting the point of an iridectomy knife into the anterior incision and along the mesial surface of the tooth, it was possible to lift upward and distally and remove the developing tooth cleanly and intact from its dental sac of connective tissue. The molar was then lifted, on the blade of the knife, and placed gently but quickly in position under the skin of the host. Silk sutures or metal clips closed host skin incisions.

Host animals were approximately 30-day-old normal healthy young adults of both sexes. Members of each series numbered from five to seven animals and were littermates. Before receiving a transplant, each host animal was anesthetized, identified by ear punches, shaved of its back fur, and an incision approximately 1 cm. long was made through the skin. With this advance preparation of the hosts, molar transplants were never more than 5 seconds from the fluids of the donor to the subcutaneous fluids of the host.

Healthy host weights at 30 days of age were found to be between 40 and 50 gm. After receiving transplants, the hosts were returned to their bank wire cages, where the regular diet of Purina pellets and water ad libitum and carrots or lettuce twice weekly was given. Animal weights at sacrifice ranged normally between 80 and 100 gm. A few hosts died or became diseased; only transplants carried by normal healthy animals were included.

After 28 days of transplant growth and development within its subcutaneous tissues, each hamster host was anesthetized, shaved, and the skin of the back incised centrally from the tail to the shoulders. After spreading the skin laterally and pinning its edges down, transplants could be readily identified. Sketch drawings were made of the general transplant outline, its color, and its vascularization. Molars, with surrounding connective tissues, were then removed and fixed in 10 per cent buffered formalin and decalcified in ethylenediamine tetra-acetic acid. Paraffin-imbedded specimens were routinely sectioned at 10 μ; a special effort was made to secure longitudinal root sections. All sections were stained with hemotoxylin and eosin.

Controls were established by comparing neonatal transplants recovered after 28 days with molars and their periodontiums which had developed normally in a 28-day-old animal.

RESULTS

The neonatal hamster molar at the time of transplantation.—A 1-day postnatal molar in situ is in the shell stage of tooth development (Fig. 1). Ameloblasts are differentiated over the cusps and are seen in earlier stages of differentiation as one passes down the slopes of the cusps either on the occlusal surface of the tooth or on the cusp slope toward the proximal of the tooth. Some dentin has been formed by odontoblasts in the central cusp, and ameloblasts here appear ready to begin enamel formation.

Bone of the jaw completely surrounds the developing molar, forming a bony crypt which is separated from the tooth by a cell-poor tissue. Osteoblasts and osteoclasts are seen along the inner surface of the crypt.

A saggital section of a molar which was removed from the jaw of a newborn donor and prior to its transplantation is shown in Figure 2. The enamel organ and dental papilla are firmer than at younger ages; at this age they are easily freed from the dental sac. Relatively few mesenchymal cells are seen outside the enamel organ. Cells seen between the cusps belong to the stellate reticulum, and the epithelium is torn and folded back at the upper left margin of this specimen.

Neonatal molar transplants after 28 days in the host.—Twenty-three neonatal molars were transplanted. Of these, 12, or 52.2 per cent, were healthy developed specimens when recovered. Gross observations of transplants were made at the time of removal from the host. Transplants which exhibited growth and development showed extensive vascularization from the host (Fig. 3). Large blood vessels supply the graft. Moreover, a capillary network can be seen spreading over its surface. Cusp outlines can also be determined. All healthy developed molars recovered were similarly vascularized and encapsulated.

The histologic appearance of alveolar bone and periodontal ligament formed in recovered specimens was similar for donors of all ages. Striking resemblances and some differences were seen between periodontal tissue developed in transplanted teeth and in teeth of similar age developed in situ. Not all transplanted teeth were recovered after 28 days. Five of 23 neonatal transplants not recovered were presumed to have been resorbed. Four transplants were recovered grossly as unvascularized, small, white, hard masses. They were microscopically dead tissues, which might have been resorbed with additional time. Two recovered transplants showed an unusual situation; grossly, they were encapsulated and vascularized, but microscopically the tissues showed little additional growth and development beyond that shown at the time of transfer. No roots were formed. Even so, bone and periodontal ligament were formed, but in these cases the periodontal tissues were found within the pulp tissue.

Figure 4 shows the histological appearance of a neonatal molar after 28 days in the host. The size and morphology of the molar were relatively normal; very little enamel formed, however. Crown dentin, not covered by enamel, was covered by cementum. Adjacent fibrous connective tissues appeared to be typical periodontal ligament; its fibers are seen to be imbedded in the cementum. Bone was formed only in relation to the cementum covering dentin of the tooth root and not in relation to the cementum and periodontal ligament over the enamel-free dentin of the tooth crown. Some cusps showed tips of dentin resembling osteodentin, in contrast to the normal tubular dentin seen above it. Roots were of normal length and contained well-vascularized pulp tissue with odontoblasts at the periphery. Pulp tissue in transplants was generally younger in appearance than in the control; it contained more cells and was more highly vascularized. Predentin was somewhat more irregular, and some areas of interglobular dentin were seen. Periodontal ligament and alveolar bone surrounded the roots; cellular cementum was heavily deposited on the sides of the roots near their apices.

Recovered transplants showed typically oriented periodontal ligament fiber groups; alveolar crest and horizontal, oblique, and apical groups could be identified. A thin

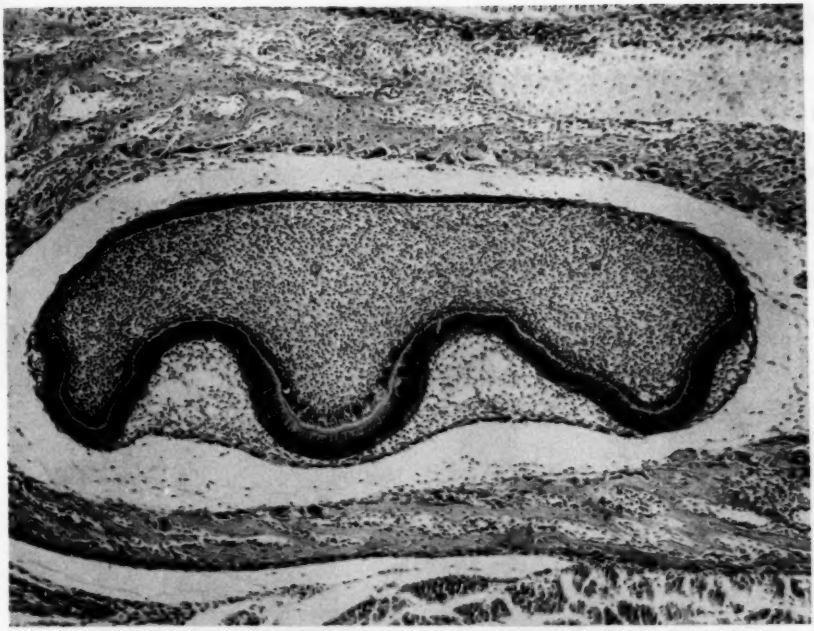

FIG. 1.—Neonatal molar in situ, saggital section. (Orig. mag. ×62.)

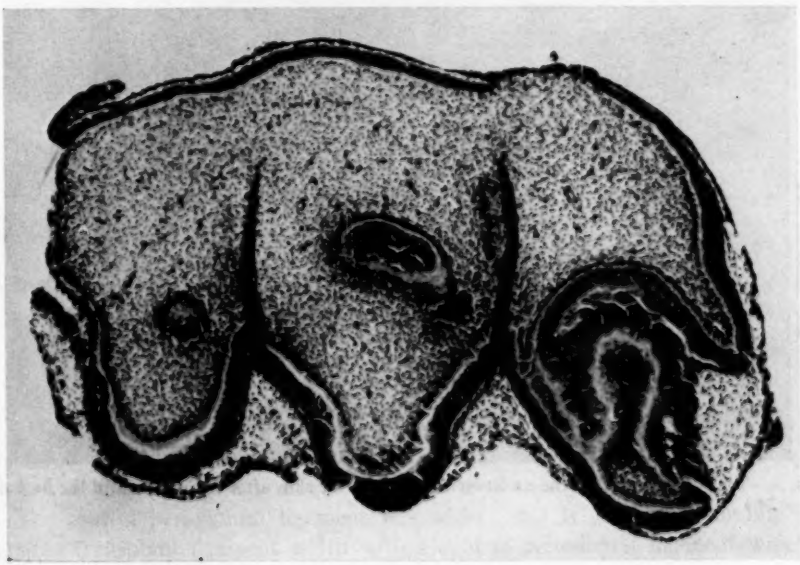

FIG. 2.—Neonatal molar removed from the jaw, saggital section. (Orig. mag. ×80.)

FIG. 3.—Transplant lying on the undersurface of the host skin after 28 days within the host. (Orig. mag. ×24.)

shell of bone surrounded the outside root surfaces, while the interradicular bone was more extensive and filled the space between the roots.

When the periodontium of the transplant (Fig. 6), was compared with the periodontium of a molar of comparable age which developed and functioned in the jaw of a control animal (Fig. 7) several points of similarity and of difference were noted.

1. Fiber orientation of the periodontal ligament between the tooth and the bone was similar in the transplant and the control.

FIG. 4.—Neonatal transplant after 28 days in the host. (Orig. mag. ×80.)

2. In contrast to the random fiber arrangement of the transplant ligament, the fibers of the control ligament were grouped into bundles which had interstitial spaces between the bundles.

3. The control periodontal ligament was wider; this is a constant finding when comparing transplant ligament width with a control periodontal ligament which developed and functioned in situ.

4. The rather delicate trabeculation of alveolar bone surrounding the transplant contrasted with the more compact alveolus of the functional control bone, with its resting lines which define prior apposition and resorption of bone. Greater cellular

activity was apparent along the alveolar bone of the control. Osteoblasts lined the osseous border of the control ligament, and an osteoclast could be seen near the upper edge of the vessel which perforates the bone.

DISCUSSION

Survival, growth, and development of transplants.—Homologous grafts have always been subject to immunological problems. These problems were expected, since random breeding was employed in our colony rather than planned brother-sister matings. Although infection may have caused the loss of a few of our transplants, genetic differences between donor and host were probably responsible for the loss of most of the transplants not recovered. Syrian hamsters, however, appear unique because they will often accept homografts of malignant tissue and occasionally even heterografts of malignant tissue as well as normal tissue. Billingham and Hellemann[8] recently tested four different closed colonies, employing random mating, for reactions to intracolony

FIG. 5.—Twenty-eight-day postnatal molar in situ, saggital section. (Orig. mag. ×80.)

and intercolony skin homografts. Intercolony survivals were 41, 17, 0, and 0 per cent for a 100-day period. The breakdown of grafts in animals rejecting intracolony homografts was complete in 3 weeks, while the median survival time of different combinations of intercolony grafts where most transplants were rejected was 10.5, 9.9, 8.8, 11.2, 19.3, and 10.5 days. It is believed, therefore, that the 28-day transplantation period used in this investigation was adequate to distinguish transplants accepted from those rejected by the host.

Vascularization of transplanted tissues is essential to their survival. In all cases it is obvious that there is an interval between transplantation and vascularization of the transplanted tissues. During this interval, transplanted tissues must acquire at least maintenance nutrition through diffusion from host subcutaneous tissue fluid. Converse, Ballantyne, and Woisky[9] showed, by grafting rabbit skin to the chorio-allantoic membrane of chicks, that transplants gained considerable weight in the early hours after transfer and before becoming vascularized. Transplant weight increased steadily from 10 per cent more after 1 hour to 52 per cent more after 20 hours. Converse calls this

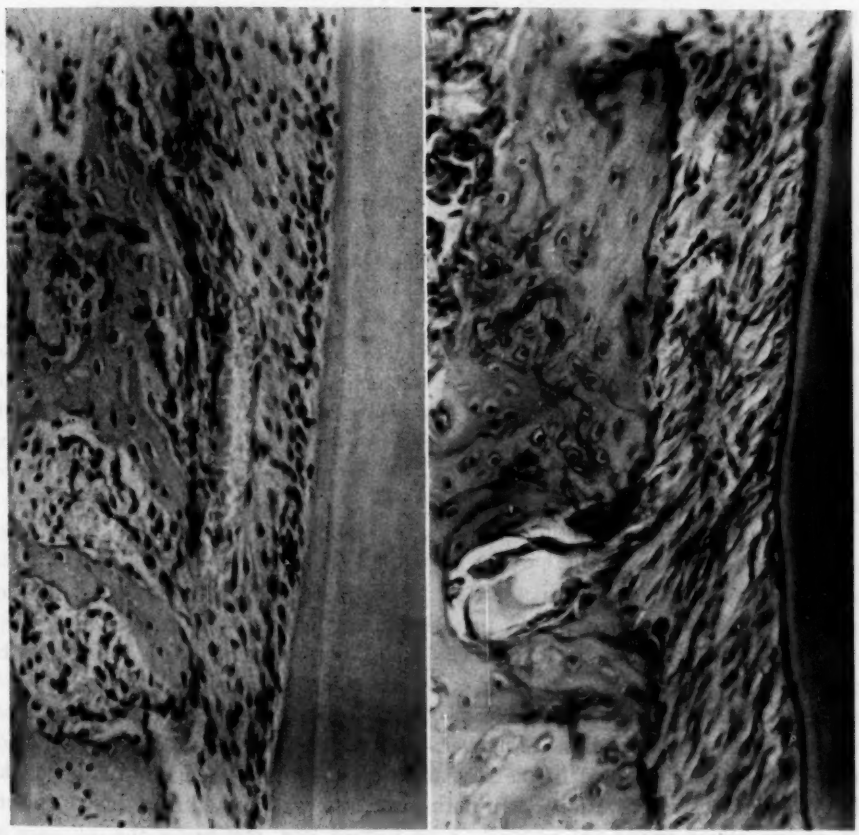

FIG. 6 FIG. 7

FIG. 6.—Transplant periodontium. (Orig. mag. ×143.)
FIG. 7.—Control molar periodontium in situ. (Orig. mag. ×143.)

initial period, during which graft vessels fill with fluid and cells from the host, the "period of plasmatic circulation." Subsequent vascularization by the host is currently being studied in our tooth transplants.

A molar which is transplanted at birth and recovered as a fully developed tooth in 28 days obviously passes through the same developmental stages as one which develops in situ. At birth, initiation and proliferation have taken place, and morphodifferentiation and histodifferentiation of ameloblasts and odontoblasts have occurred. The size and shape of the tooth crown are established, and crown morphology is made permanent by the beginning of dentin formation at the dento-enamel junction. Following transplantation, apposition and calcification of enamel and dentin occurred, as well as the formation of roots and periodontium—processes which are normally associated with the eruption of the tooth. Hertwig's sheath also survives transplantation (Fig. 8).

Organization of transplants.—Figure 9 is a diagrammatic representation of the experiment. The developing germ and its adjacent tissues—the lower center figure—forms the fully developed molar and its periodontium—the figure on the right. It is seen that the ectodermal derivative—the enamel organ—*A* forms the enamel *A′* of the fully developed tooth. Mesodermal elements—the dental papilla—*B* formed dentin *B′*, and the dental pulp *B′*. Connective tissue *C* adjacent to the tooth forms the periodontal ligament, *C′*, and adjacent bone, *D*, forms the alveolar bone, *D′*, which supports the developed tooth.

However, when the developing tooth is transplanted to the new connective tissue, as shown in the lower center to upper center figures, only *A*, *B*, a very small amount of *C*, and none of *D* are transferred to the new environment, *E*. The transplant develops into the mature tooth and its periodontium at the left. *A* develops into *A′*, *B* to *B′*, *C* may possibly proliferate to *C′*, but bone was not transplanted, and yet a shell of bone formed around the roots of all healthy recovered transplants.

We have seen that if the transplant survived and developed, it formed periodontal ligament and alveolar bone around its roots. This seems to indicate that the morphologically complete dental organ consists of the enamel, dentin, pulp, cementum, periodontal ligament, *and a shell of alveolar bone surrounding the roots*. Teleologically speaking, transplanted teeth obviously considered themselves incomplete without this bone and hence caused it to be formed. No healthy developing molar failed to produce it.

The formation of periodontium around transplanted enamel organs may be analogized to the induction of the lens by the optic cup. Like the developing tooth, the forming eye is composed of structurally discrete parts which originate from different embryonic sources but are brought together during development to form a complete organ. Spemann[10] points out that Lewis found that when the early rudiment—the optic vesicle—of the frog eye (*Rana sylvatica* and *palustris*) was exposed, severed, and pushed backward under the epidermis of the trunk, a lens formed above the trunk from the epidermis of the host. Moreover, after the eye was fully formed, removal of the lens in *Urodele* resulted in the formation of a new lens from the border of the iris (Wolffian lens regeneration).[11] In both cases, lens was formed from tissue, epidermis, or iris, which would not normally have formed lens, and under the influence of a stimulus provided by the optic cup. The eye was "incomplete" without lens and hence proceeded to acquire one. Similarly, the transplanted germ was an incomplete

FIG. 8.—Apical area of neonatal transplant after 28 days in the host. (Orig. mag. ×135.)

developmental unit and completed itself during its development by producing periodontal ligament and alveolar bone.

We may now see the formation of periodontal tissues around transplanted molars as the efforts of a morphogenic field to complete itself. The development of other morphogenic fields, when transplanted, has been shown to be similar in pattern to the development in situ.[11] This suggests that the development of the transplanted molar is also the *normal* developmental pattern. Developing 6-day postnatal hamster molars show a shell of alveolar bone which is not completely attached to the jaw in some areas. This appears to support the contention that the mode of development of the transplant and the molar in situ are similar.

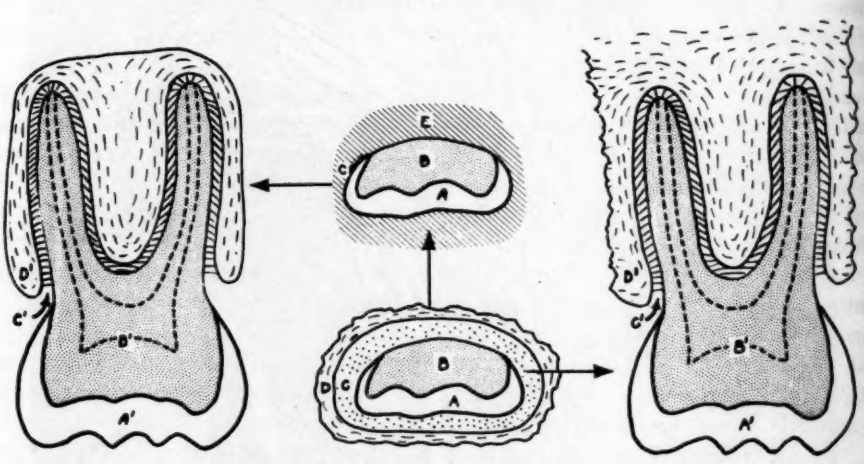

FIG. 9.—Diagram of the experiment. Figure on left shows transplant after development in host; figure on right shows fully developed molar and its periodontium in situ. A = enamel organ, A' = enamel of fully developed tooth; B = dental papilla, B' = dental pulp; C = connective tissue, C' = periodontal ligament; D = adjacent bone, D' = alveolar bone; E = new environment.

The source of cells which form bone.—Most workers agree, perhaps because of lack of other evidence, with Tomes' statement as reported by Mummery, that "the follicle is in fact formed by a condensation of the connective tissue in the neighborhood of the tooth germ."[12] It now appears clear that the cementum, periodontal ligament, and alveolar bone shell are the derivatives of the dental sac.

The cells which form alveolar bone around transplants could have come either from transplanted tissues, the enamel organ, dental papilla, adhering connective tissue cells, or the new environment which lacked the bony crypt of the jaw. It seems quite acceptable that the enamel organ, A, should again form enamel, A', and that the dental papilla, B, should form the dentin and pulp tissue, B'. However, according to Figure 9, several possibilities exist as to the cell origins for the periodontal ligament, C', and especially for the shell of alveolar bone, D'. Only a small part of C and none of D were transferred. Moreover, the new environment, E, must be evaluated as to its possible contribution.

The enamel organ, A, is a specialized epithelial derivative of ectoderm. The possibility that such specialized epithelial cells might be converted to cells forming the

mesodermal connective tissue—bone—seems remote. The enamel organ is therefore eliminated. Connective tissue cells from the dental papilla, *B*, could have proliferated and migrated out of the inferior lateral corners and surrounded the tooth, as originally proposed by Mummery[12] and Hopewell-Smith.[13] If this were true, then these cells of the papilla would be expressing a potential greater than their normal prospective significance of forming odontoblasts and miscellaneous pulp cells. The possibility that the adhering cells, *C*, could proliferate and be converted into the bony shell is granted. It is also possible, however, that the cells of the subcutaneous connective tissue environment which were not intended to form periodontal ligament and alveolar bone were converted, under the stimulus of the developing tooth, into cells which formed these tissues. This hypothesis implies the conversion or modulation of cells into osteoblasts.

The literature of experiments related to heteroplastic bone formation indicates that a number of different connective tissue cell types have been shown to be able to be modulated to osteoblasts to form bone. The controversy still exists as to whether the fibroblast can be converted into an osteoblast; the opponents maintain that an undifferentiated mesenchymal cell is a more likely candidate for conversion to an osteoblast than is the more differentiated fibroblast. Under any circumstances, there were ample cells which, although outside normal skeletal areas, became capable of forming bone.

Stimulation for bone induction.—In placing the location of the osteogenic stimulus capable of influencing the conversion of connective tissue cells to osteoblasts, we may consider that, since bone is formed around the roots of developing teeth in situ, the stimulus may originate in either the developing tooth (intrinsic) or from outside the developing tooth (extrinsic). This assumes only one stimulus originating from one location. The transplanted developing tooth formed periodontal ligament and alveolar bone in a connective tissue site different from the normal one—one which does not ordinarily form bone. Therefore, the stimulus must be located in the transplanted tissues. From which transplanted tissue does the stimulus come, the enamel organ or the dental papilla?

The enamel organ is the epithelial portion of the developing tooth. Component parts of the enamel organ are the outer enamel epithelium, the stellate reticulum, stratum, intermedium, and inner enamel epithelium. After formation of the crown of the tooth, the inner and outer enamel epithelium are believed to join in the formation of Hertwig's sheath, which guides and serves as a mold for root formation.[14, 15] As a developing unit, the enamel organ has been reported to exert a dominating influence over the condensation of adjacent connective tissues which form the dental papilla and dental sac.[15] The inner enamel epithelium differentiates into tall columnar cells—the ameloblasts—which have been shown to influence the adjacent connective tissue cells of the dental papilla to differentiate into odontoblasts.[14] Moreover, after enamel formation is complete, the columnar ameloblasts join the stratum intermedium and outer enamel epithelium to form a flattened squamous epithelial layer. This reduced enamel epithelium is reported to have the capacity to induce atrophy of the connective tissues separating it from the oral epithelium.[15] Since in four instances the influence and control of the epithelium of the enamel organ upon surrounding connective tissues seems certain, it may be reasonable to suggest that the epithelial derivative—Hertwig's sheath—is also able to influence the surrounding connective tissues to form perio-

dontal ligament and alveolar bone. The fact that bone forms normally only when roots are formed and only in relation to these roots seems also to point to the root sheath as the origin of the stimulus.

Lefkowitz, Bodecker, and Shipiro[16] experimentally removed the dental papilla, including odontoblasts, from developing mandibular cat canines in situ. They found that the various mesodermal tissues which filled the space created by the removal of the papilla assumed an appearance closely resembling a normal periodontium. Structures similar to cementum, periodontal ligament, and alveolar bone were observed in the pulp area. Therefore, if the papilla can be removed from developing teeth in situ and if periodontal tissues are formed both in replacing connective tissues and around the root, the stimulus for periodontal tissue formation must be the enamel organ and its derivative—Hertwig's sheath.

The completely intact tooth germ is not ordinarily subjected to pulpal changes resulting in structures characteristic of the periodontium. However, Santone[17] punctured developing guinea-pig tooth germs with a sterile needle and observed the responses of the tooth in healing. If the germ was punctured before dentin had been formed (bell stage), fibrous connective tissues typical of periodontal ligament filled the hole caused by the injury. In our experiment, two transplanted newborn molars which survived but did not develop roots had a demonstrable break in the inner enamel epithelium. In these specimens the dental papilla became more fibrous, with islands of bone both free and attached to the dentin surfaces. The free islands of bone are parallel to the predentin, and tissue resembling periodontal ligament is seen between the bone and the predentin. In the Lefkowitz case, total replacement of the papilla was made by tissues which resembled periodontium. In Santone's case, partial revision of the pulp to tissue resembling peridontium was effected by a small penetrating injury, while, in the case of the transplanted molars, total pulpal revision appeared due to a tear in the inner enamel epithelium. It seems possible that the various invasions of the papilla permitted the entrance of the postulated stimulus into the pulpal areas, which were then revised to tissues resembling periodontium under the influence of the stimulus.

The proposal that a proliferating epithelium may stimulate bone formation is not new. Efforts to recognize and identify a substance capable of converting undifferentiated mesenchymal cells and/or fibroblasts to osteoblasts in extra-skeletal areas has aroused great interest since Huggins[18] demonstrated the ability of transplanted urinary bladder epithelium of dogs to stimulate adjacent connective tissues to form bone. Several investigators have subsequently confirmed Huggins' original finding. This is clearly bone induction. However, the nature of the stimulus and the mechanism through which it operates are unknown.

No author has denied the existence of a substance capable of inducing bone formation. However, to date, no author has made positive identification of an active ingredient in an acid alcohol of any other extract which is osteogenic when injected.

It is possible that the enamel organ and Hertwig's sheath of the developing tooth produce such a substance. In this respect, it seems that the developing tooth falls into Urist and McLean's[19] group of tissues which form new bone by stimulating host connective cells to be converted into osteoblasts. However, additional evidence should be obtained to establish conclusively that no transplanted cells were precursors to perio-

dontal tissues formed. Without doubt, a certain few cells adhered to the outside of the outer enamel epithelium and were transplanted. It seems improbable that these few cells could have been responsible for the extensive formation of periodontium routinely seen around transplanted teeth after 28 days in the host subcutaneous tissues. More likely, the cells forming the periodontium were host cells, which, under the influence of the enamel organ and Hertwig's sheath, were converted to cells capable of forming the various elements of the periodontium.

Stimulation of function.—When comparing periodontal ligament formed by transplants and by molars developing in situ, one notes the striking similarity in the appearance of the fibrous elements and also that similar cells—fibroblasts, cementoblasts, osteoblasts, and osteoclasts—are seen in both. Similar structures have similar positions. Blood vessels have the same relationship to bone of the alveolus and to the periodontal ligament. The arrangement of fibers in the periodontal ligament of the transplanted molars is of particular interest. Gingival fibers, alveolar crest, and horizontal, oblique, and apical fibers are found in identical root areas as those in the control molar which developed its periodontium in situ. This is also true of periodontal ligament developed from teeth transplanted both prenatally and postnatally.

However, some differences also exist between periodontal ligament developed in the absence of function, the transplants, and the controls which were exposed to functional influences during development. Fewer blood vessels were found related to the periodontal ligament of transplanted molars. Fiber-bundle definition is less clear in periodontal ligaments which have never been functional. Moreover, there is less grouping of fibers into bundles and consequently indefinite interstitial spaces (Fig. 7). Although comparative central sections have not been subjected to precise measurement, it is obvious that the periodontal ligament of transplanted molars is narrower than in the molar which develops with function in situ. Some transplanted molar periodontal ligaments show only a few fiber bundles between the middle of the root and the apex. A few long fibers are seen running parallel to the root surface in these areas.

These similarities and differences between functionless and functioning periodontal ligaments seem best understood on a biological basis. Wilhelm Roux, as quoted by Russell in his book *Form and Function*,[20] first established the concept that there were two periods of development. The first period was under the control of genetic factors inherent in the germ plasm, and the second—the further differentiation and maintenance of form—was controlled by functional activity. This concept appears directly applicable to this study, since transplantation ostensibly separated the development related to genetic factors from that related to function, by providing for development in the absence of function. Within the first period, Roux includes all growth, development, and maintenance of an organ that take place without active function. Then, with function, there is an additional formative activity which acts partly by producing new form and partly by maintaining that which is already formed. This he calls the "finer functional differentiation of the organ."

Subsequent examples of the effect of function upon structure, which have placed more emphasis on the genetic factors operative during the first phase of development, have been shown. The structure of muscle, for example, has been shown to be completely developed without function. Hamburger, quoted by Weiss,[21] prevented the innervation of developing limbs and found normal muscular development despite the

elimination of all function. However, he also found that, unless the muscle was innervated and functioned before a certain critical stage, some of the earlier differentiations were eliminated by regressions in structure. It was apparent that muscle could develop without innervation and function but, once developed, required them if it were to be maintained.

By culturing explanted fowl limb buds in vitro, Fell and Canti[22] showed that bones and joints could also form typically without movement. However, fusion of the bones took place later because of the lack of movement. Again normal structural features were not maintained without function.

It is also known that the internal trabecular pattern of the long bones of the embryo is structurally preadapted for function before any body weight has been placed upon them. In this case, however, Weiss[21] states that function itself can mold such patterns directly, particularly after injury, during later stages of life.

It seems probable that fiber orientation of the periodontal ligament is similarly developmentally preadapted and does not require function in the formation and orientation of its fibers. This assumption is substantiated by the fact that transplanted molars developed typically oriented principal fibers without the influence of function. One might argue that a certain amount of movement of the transplant beneath the host skin might serve as adequate function. While this possibility exists—and if it were true—the function required to affect the orientation of fibers would be far less than masticatory forces. Moreover, transplants were grossly oriented in all directions within the host, and yet the fiber orientation was similar in all.

How, then, are the differences in the non-functional and functioning periodontal ligament to be accounted for? Preissecker[23] removed the upper or lower molars on one side in a group of rats, to determine the effect of lack of function upon the periodontal ligament of the unopposed remaining molars. When these were later compared with molars on the opposite side of the mouth where function remained, he found their periodontal ligament width to be approximately one-third less than in those molars which had been in occlusion. Function, then, was responsible for a thickening of the periodontal ligament.

Gottlieb[24] called the minimum width of the periodontal ligament between the root and the alveolar bone, as represented by the unerupted and imbedded tooth, the "biological periodontal width." He referred to the larger functional width as the "physiological periodontal width." It seems that the biological periodontal width is represented by the periodontal ligament formed around transplanted molars. In order to characterize this ligament further, the term "biological prefunctional" is suggested —"biological" to indicate the minimum width and "prefunctional" to indicate the preadaptation of structure to its future functional requirements.

Teeth which are subjected to normal function have strong, well-developed fiber bundles (Boyle).[25] Non-functioning teeth lack this definite fiber-bundle formation. It seems probable, therefore, that the lack of prominent fiber bundles in the periodontal ligaments of the transplanted teeth represents the lack of functional influence in modifying the structure of the ligament. Boyle also states that the periodontal ligaments of unopposed and imbedded teeth are sometimes replaced by fat tissue. Apparently, function is as important in the maintenance of the periodontal ligament as it was in the preservation of muscle, joints, and bone.

In summary, the periodontal ligament development is believed to be essentially prefunctional and preadapted in its structure and principal fiber orientation. Function modifies the biological prefunctional ligament by increasing its width and grouping its fibers into more definite bundles. These modifications are believed to be functional adaptations important in the maintenance of the tissue. This concept seems to fit well with Roux's original and Weiss's later suggestion that the development of most organs passes through a prefunctional formative phase and that subsequent functional activity may modify these structures slightly in the conservation or maintenance phase. Each phase, they state, is under the prevailing control of a different set of factors, the first phase controlled by genetic considerations and the second by functional factors.

SUMMARY AND CONCLUSIONS

1. Developing hamster molars prior to periodontal tissue formation were transplanted subcutaneously into 30-day hamsters to determine their capacity to develop periodontal ligament and alveolar bone in a connective tissue environment other than the jaw. Tissues transferred included the enamel organ, dental papilla, and a certain small number of cells which cling to the external surface of the external enamel epithelium; no bone was transplanted.

2. If the transplant survived and was vascularized by the host, it formed typical periodontal tissues and a shell of alveolar bone as integral parts of the complete dental organ.

3. The development of the dental organ, the tooth, its periodontal ligament, and a shell of alveolar bone appears to represent a morphogenic organ field. Therefore, biological principals such as "wholeness," organizer activity, and the competence of tissue, should be applicable to the developing tooth and its periodontium.

4. The original jaw bony crypt surrounding the tooth germ was not transplanted. Therefore, the source of cells which formed bone was provided either by one of the transplanted tissues, the enamel organ, dental papilla, or adhering cells, or by the tissues of the new connective tissue environment.

a) The enamel is presumed not to be the source of cells forming the connective tissue periodontium, since it is entirely epithelial.

b) If the cells forming the periodontium came from the dental papilla, then proliferation and migration occurred, and these cells realized a potential greater than their normal prospective significance.

c) Mesenchymal cells adhering to the outer enamel epithelium may have proliferated.

d) If the new environment supplied the cells which formed the periodontium, then cells not ordinarily forming bone were modulated or differentiated into osteoblasts.

5. The source of the stimulus for the formation of periodontal tissues obviously resides in the tranplanted tissues. It is proposed that, of these tissues, the enamel organ and its derivative—Hertwig's epithelial root sheath—are the most likely source of the stimulus.

6. A typically functional orientation of periodontal fibers is found in recovered transplanted teeth which have not functioned as in mastication. Basic fiber orientation appears to be developmentally prefunctional and preadapted, while the grouping of fibers into bundles and the increase in fiber detail seem to represent modifications of structure to function associated with the maintenance of the periodontal ligament.

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Studies on the Innervation of Human Teeth

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Ache and pain in or near the teeth and disturbances in their nerve supply are matters of the utmost importance in odontology. They are also significant problems in modern otorhinolaryngology—the otologist is often consulted for earache or facial pain when the patient's trouble actually is of dental origin. Accordingly, the innervation of the human tooth is a subject of common interest to the otologist and the dentist. And in modern hospitals these specialists regularly collaborate when a patient's dental nerves are injured or otherwise affected. Furthermore, it is becoming an increasingly frequent practice to use the teeth as test organs for trying out and evaluating new anesthetics and analgesics. This side of the question thus provides another reason for the urgency of acquiring as accurate information as possible about the innervation and sensitivity of the teeth.[1-4]

The question of dental innervation has recently been discussed and studied by Arwill,[5] to whose extensive survey of the literature the reader is referred. He concluded that many of the investigations by previous workers suffered from defects of various kinds. His most important reservations considered the risk of generalizing findings from test animals only and not from human beings. Furthermore, some authors "claim to have found a specific nerve staining method," and he stated that, "as long as the selectivity of the method remains unproved, the results obtained by this method should be evaluated with reservation." As a third factor he mentioned that only few studies had been performed with sufficiently thick sections to retain the continuity of the nerve bundles for identifying nerve fibers.

Should we seek to acquire, by reading, a general idea of the innervation of the teeth, we should have to peruse a vast literature full of contradictory statements and unsolved problems. The density of the innervation in various dental regions, the association between the nerves supplying the deciduous teeth and those in the permanent teeth, the general topography of the nerves which lead to the permanent teeth from different points in the jaws, and how the nerves from separate root canals of premolar and molar teeth are involved in the innervation of the coronal pulp are some of those problems about which we know little or nothing. And, although a good many workers have studied the fate of the nerves when a tooth is lost or damaged, our knowledge is far from complete.[6-12]

The main obstacle to the study of dental innervation is the technical preparation of the dental pulp in its hard shell of dentin and enamel. Most researchers have used decalcified and sectioned teeth or sections of pulp removed from split teeth. Very few have studied the pulp as a whole.[13, 14]

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For the purposes of the present investigation it was deemed necessary to acquire a rough idea of the morphology of dental innervation insofar as it remained in doubt. One of us (A. Ö.) is at present in the midst of a more extensive investigation into the innervation of human teeth and the extra-dental course of the nerves supplying the teeth. As the work has progressed, it has become apparent that its aims cannot be realized unless previous methods are improved and new ones developed. The present paper will be concerned mainly with such new methods but will also briefly recount some of the results so far obtained by their use.

MATERIALS AND METHODS

The material for this study was 105 human dental pulps from unerupted or impacted teeth or teeth that had been in use for varying lengths of time. Complete absence of caries was the one common feature. Grounds for extraction varied; most

TABLE 1*

DISTRIBUTION OF THE MATERIAL IN RELATION TO AGE AND LOCALIZATION OF THE TEETH

Tooth Number	Amount of Teeth by Age			Tooth Number	Amount of Teeth by Age		
	<16 Yr.	16–40 Yr.	>40 Yr.		<16 Yr.	16–40 Yr.	>40 Yr.
+1+	.....	2	.....	−1−	1	.....	.....
+2+	1	.....	.....	−2−	.....	.....	.....
+3+	6(5)	11(8)	3(2)	−3−	.....	2	.....
+4+	6	2	.....	−4−	7(1)	1(1)	.....
+5+	13(8)	2(2)	.....	−5−	8(6)	.....	.....
+6+	7	1	.....	−6−	4	1	.....
+7+	2	.....	.....	−7−	1	.....	.....
+8+	.....	4(1)	.....	−8−	.....	9(4)	.....
Total	35(13)	23(11)	3(2)	Total	21(7)	13(5)	.....

* The material also includes 3 supernumerary and 7 deciduous teeth. The figures inside parentheses refer to the number of impacted teeth in the material.

of the teeth had been removed because of irregularity or for cosmetic reasons, some had been removed prior to fitting artificial dentures, a few had been loose, and a small number had had to be extracted after accidents. Hence it is only to be expected that most of the teeth came from fairly young persons. In Table 1 the teeth have been classified according to age and type.

Dental pulp dissection and fixation.—In order to minimize the interval from the interruption of the pulpal blood supply owing to dislocation of the tooth to the immersion of the pulp into the fixation agent, it proved helpful to adopt a standardized preparation schedule, which was varied slightly, depending on whether the pulp was intended for examination under the light microscope or in the electron microscope.*

In cases in which the pulp was destined to be examined electron-microscopically, very careful arrangements were made to facilitate extraction of the tooth. Any alveolar bone that might prevent rapid extraction was removed, and attempts to pry up the tooth with an elevator were avoided, lest its circulation be interrupted prior to the

* RCA, model EMU 3.

exact moment of lifting it out of its socket. With the aid of a diamond wheel, a groove was ground in the desired cleavage plane of the enamel and cementum of the tooth immediately after its extraction. To prevent generation of excessive heat, this was done under a cold-air blast. The tooth was then split with a vise provided with two pairs of sharp edges, made especially for the purpose (Fig. 1). Whereas incisors, cuspids, and bicuspids most conveniently were split from the apical end, molars generally proved easier to deal with when a large portion of the crown was first split off by cleaving the tooth at right angles to its long axis and then cracking open each root separately. After the pulp had been thus dissected free, it was at once immersed in veronal-buffered 1 per cent osmium tetroxide solution (according to Palade[15] and Sjöstrand[16]).

When the pulp was intended for examination under the light microscope, the tooth was split, in order that the fixation fluid would be capable of rapidly permeating into the pulp, which was dissected after it had been fixed in the osmium tetroxide solution or in 10 per cent formalin solution. Formalin-fixed pulps were also placed in the osmium solution but were first dissected free from hard substances.

The routine procedures for Golgi staining and methylene blue staining described in any histological handbook were employed.

Practically all previous workers have studied the innervation of the pulp after paraffin-bedding and sectioning in the usual way. In the light-microscopical part of the present investigation, on the other hand, our aim was to retain the pulp as an undivided unit and to visualize simultaneously all its innervating structures. It was found that under a stereoscopic dissection microscope the nerve fibers—stained black by the osmium treatment—could be manifested in a three-dimensional picture, if the surrounding pulpal substance could be made invisible by bedding the specimen in a medium with a suitable refractive index (in our material, usually immersion oil). Throughout the length of pulps bedded therein, one could often trace small bundles of nerve fibers or even single fibers. This technic thus enabled us to observe in great detail the complex pulpal innervation. With knowledge of the over-all appearance of the innervation, more minute studies could profitably be made by microdissecting separate nerve bundles and following their branches, or by examining thick or thin serial sections of a pulp that had been paraffin-bedded after being studied as a unit.

Specimens intended for electron-microscopic observation were bedded in methacrylate and sectioned in the usual manner. We found Morán's diamond knife very helpful for cutting the sections, and no special precautions were required to preserve the pulp from damage by dentin particles.

General morphology of dental innervation.—The adopted technic with osmium impregnation or methylene blue staining enables both coarse and fine nerve branches to be followed under the microscope throughout their course in all parts of the pulp. It was found that the appearance of the pulpal ramifications of the nerves entering the tooth was highly dependent on the number of root canals. We therefore found it convenient to classify the teeth by the number of their root canals.

RESULTS

Teeth with one root canal.—At the apical foramen, the myelinized nerves, as a rule, included some coarse trunks. These were generally accompanied by several appreciably

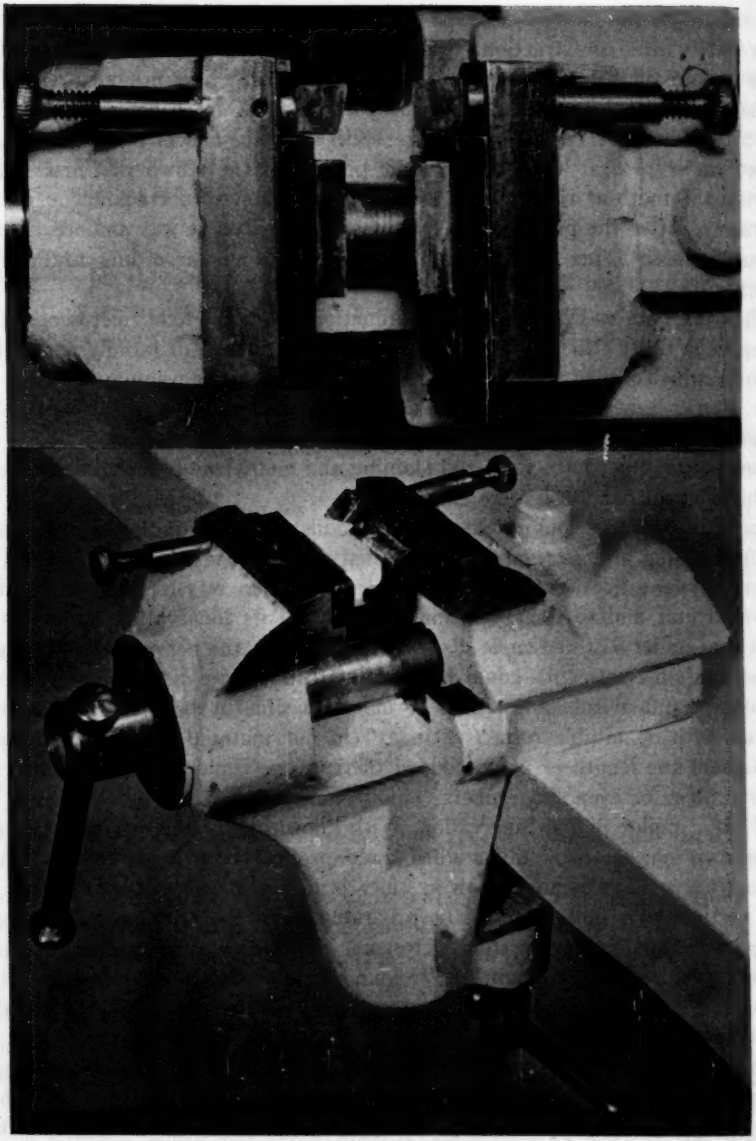

FIG. 1.—Device for the splitting of the teeth, consisting of a screw-vise provided with two pairs of cutting or breaking edges. One pair of the edges can be separately adjusted to fit the form of the tooth. By turning the main screw, forces are thus applied at the same time to different parts of the tooth.

smaller nerve bundles composed of myelinized fibers in varying numbers, often as few as 20 or 30. From the tip of the root to the coronal pulp the incoming trunks usually took a straight course and gave off few branches (Fig. 2). Very occasionally, small branches issuing from the main trunk could be seen in the pulp canal, which, however, appeared to be predominantly innervated from the narrower trunks surrounding the coarse ones. But, compared with the extremely profuse ramifications encountered in the coronal pulp, the density of secondary nerve branches seemed to be considerably

FIG. 2.—Myelinized nerve bundles inside the root- and crown-pulp

lower in the root than in the crown. On reaching the coronal pulp, most of the incoming nerve trunks could be seen to ramify profusely, and this part of the pulp was pervaded by an intricate network of interwoven nerve branches of different sizes, which here and there anastomosed. The nerve fibers seemed especially dense in the so-called horns of the pulp, and the branches innervating corresponding horns could almost invariably be traced to the same incoming trunks, indicating that the dental pulp was innervated in accordance with a predetermined fixed pattern. Among the nerve branches, particularly those in formalin-fixed pulps, a dense vascular plexus, in which the largest vessels usually lay nearer the center of the pulp in both crown and root, was observed. The coarse nerve trunks were often disposed alongside entering blood vessels with fairly thick walls—probably arterioles—but the venules exhibited no

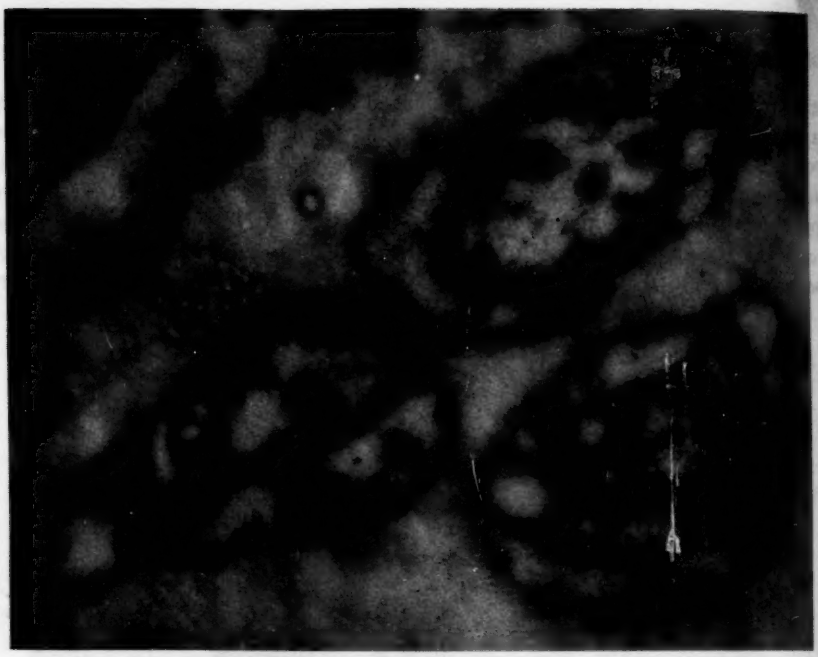

FIG. 3.—Unmyelinized nerve fibers from the apical portion. (Impacted upper cuspid from a 52-year-old woman.)

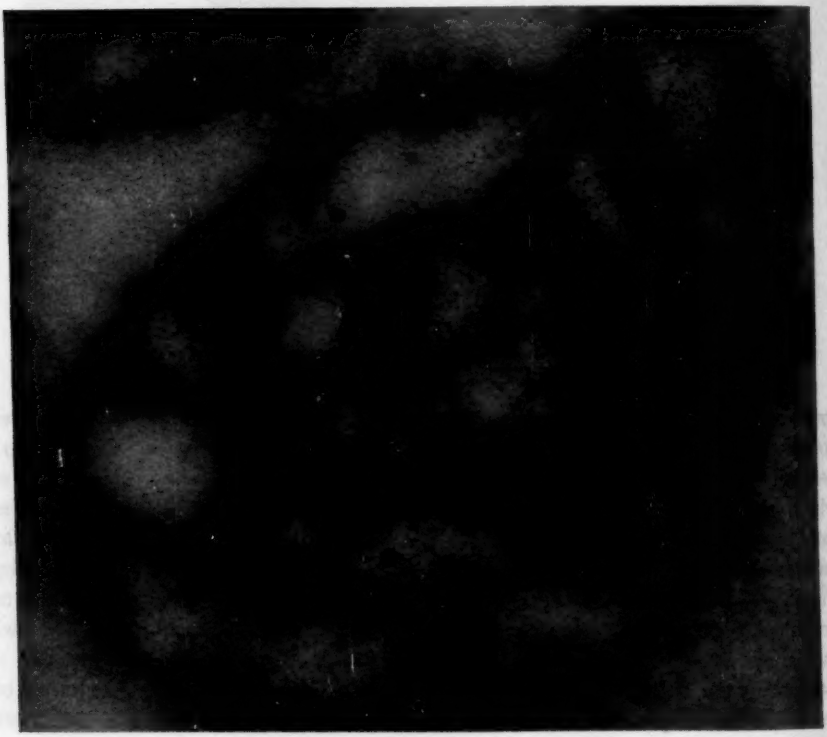

FIG. 4.—Detail from the preceding Fig. 3

obvious regularity in their arrangement. In the coronal pulp the capillaries were particularly abundant near the odontoblast layer, and whole-pulp specimens consequently gave the impression of having a finely meshed network of capillaries and thin nerves beneath the odontoblastema. The number of ingoing nerve fibers varied within wide limits.

Since osmium impregnation affects only myelinized nerves, methylene blue staining and electron microscopy were used to supplement the observations made in osmium-impregnated specimens. These procedures disclosed the presence in the root canal of

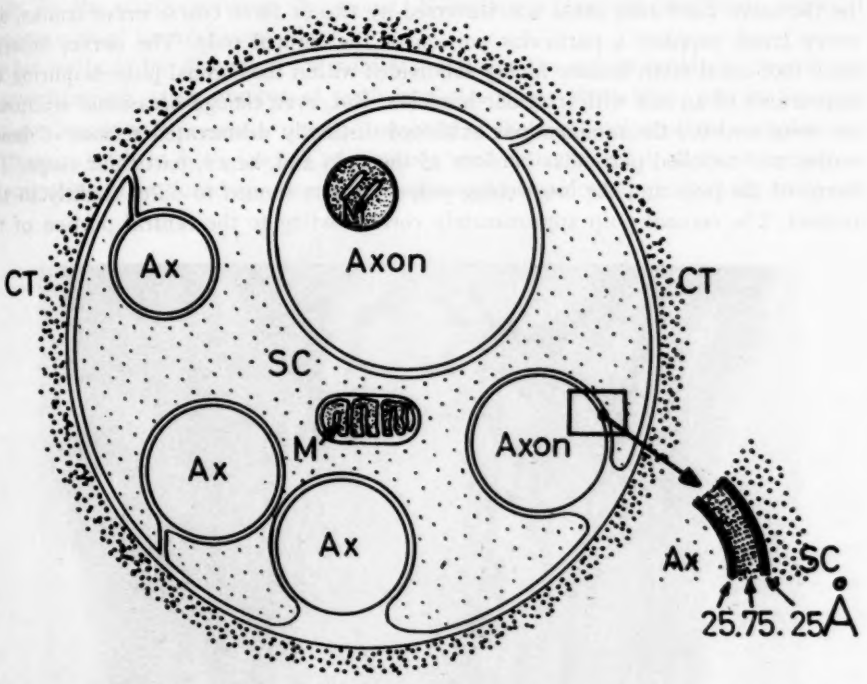

FIG. 5.—Schematic drawing of an unmyelinized nerve-fiber group. *Ax* = axon; *SC* = Schwann cell; *M* = mitochondrion; *CT* = connective tissue.

bundles of unmyelinized nerve fibers surrounded by sheath cells. They lacked the characteristics of terminal unmyelinized nerve fibers and probably represent an autonomic innervation (Figs. 3, 4, 5). These delicate, unmyelinized fibers were often disposed near or alongside the walls of the blood vessels and occasionally lay coiled around the vessels. They were also distributed among the myelinized nerves, irrespective of whether vessels were present.

Teeth with two root canals.—As a rule, one or two thick bundles of nerve fibers and several smaller bundles entered the tip of each root of these teeth. As in teeth with a single canal, they took a fairly straight course through the pulp in the root canal and gave off few branches, the root pulp predominantly being innervated from the small bundles. On reaching the coronal pulp, the large bundles ramified and formed

a rich plexus, although particular horns of the pulp always were supplied from the nerves entering the same root. In these teeth, comparatively large nerve branches approached fairly close to the horns before ramifying. The two root pulps of some teeth in our series were connected by a thin membrane, which could be clearly distinguished all the way from the apices to the coronal pulp. In the root septum thus formed (Fig. 6), one could even perceive separate fibers in the small entering branches (Fig. 7).

Teeth with three or more root canals.—As in teeth with two root canals, nerve-fiber bundles entered all the apices, and their course through the root canals appeared to be the same. Each root canal was traversed by two or three coarse nerve trunks, and every trunk supplied a particular portion of the coronal pulp. The nerves entering each root canal often became highly subdivided within the coronal pulp, acquiring the appearance of an oak with irregular branches. But, even though occasional anastomoses were noticed, the ramifications exhibited distinctly demarcated regions of innervation and supplied particular portions of the pulp and, hence, particular cusps. The horns of the pulp and the intervening pulpal regions seemed to differ slightly in this respect. The coronal pulp approximately corresponding to the central portion of the

FIG. 6.—Pulp from a bicuspid tooth

masticating surface was penetrated by relatively coarse nerve branches, which extended almost all the way to the pulpodentin junction before sprouting out like a fountain on this junction. These fountains or broomlike tufts were highly characteristic.

DISCUSSION

The pulp was found to be profusely innervated in all the 105 teeth examined, and in most of them the entering nerves exhibited a characteristic course. The pulp was pervaded by two distinct types of nerve fibers—myelinized and unmyelinized. The latter were probably autonomic and supplied the dense vascular network of the dental pulp. In the sciatic nerve of the mouse, Robertson[17] observed practically identical appearances of unmyelinized fibers.

Our methods for dissecting the pulp and staining the nerves enable us to examine a comparatively large number of teeth in a reasonably short time; and in the near future Öhman will present a detailed analysis of the innervation of the complete set of human teeth in both jaws. The methods used hitherto, most of them leading to examination of sectioned specimens, are tedious and occasionally misleading, while our

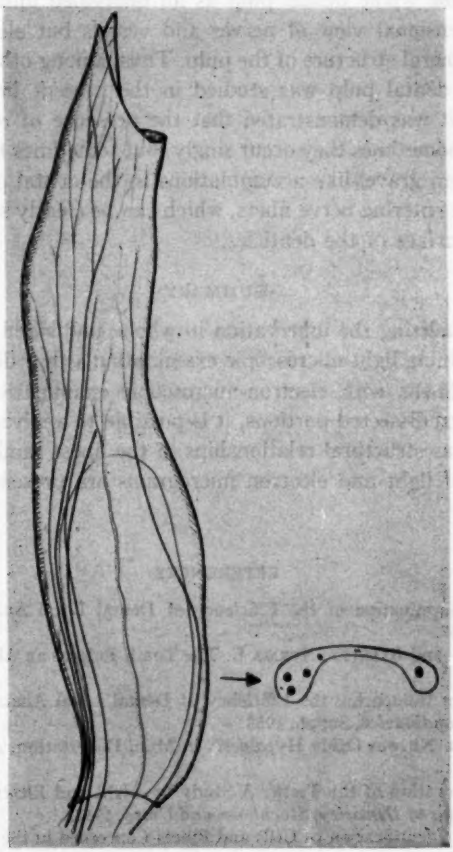

FIG. 7.—Schematic drawing of a pulp with a thin septal portion in the root

technic clearly visualizes the entire course through the pulp not only of coarse nerve trunks but also of separate nerve fibers. It will make it possible to analyze dental innervation during the shedding period and during odontogeny and to observe nervous changes associated with pulpitis and other pathological conditions. Everything from gross appearances to the minutest details of pulpal innervation can readily be studied by using combinations of the methods described here. Such an investigation is in progress, and representative appearances ranging from coarse nerve trunks to single, seemingly autonomic fibers are illustrated in Figures 2, 3, and 4.

The dental pulp exhibits a remarkably high nerve density, as a number of previous workers have also pointed out and variously explained.[7, 10, 12, 18-20] The reason might be that evolutionally the teeth may be regarded as invaginations and consequently represent a large surface. While the existence of a rich dental innervation within a shell of enamel and dentin, of course, might lack functional significance, it could just as well serve a functional purpose and enable our teeth, via the pulp and thence centrad, to mediate nerve impulses associated with the act of mastication and the intake of food.

Examination of the whole dental pulp as an undivided unit not only provides an excellent three-dimensional view of nerves and vessels but also yields certain information about the general structure of the pulp. Thus, among other things, the existence of denticles in the dental pulp was studied in the present investigation; and, as a subsidiary finding, it was demonstrated that the presence of round or oval denticles is a common thing. Sometimes they occur singly, but sometimes they are very numerous and occasionally form gravel-like accumulations in the dental pulp. These formations are enmeshed by the entering nerve fibers, which can be clearly seen lying like flattened ribbons along the surface of the denticles.

SUMMARY

A method for visualizing the innervation in whole, undivided dental pulps has been developed. By combining light-microscopic examination at low degrees of magnification of whole-pulp specimens with electron-microscopic examination at high degrees of magnification of microdissected portions, it is possible to analyze the total innervation of the pulp, as well as structural relationships in the finest ramifications of the dental nerves. A number of light and electron micrograms are presented in support of this claim.

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Infrared Spectral Characteristics of Pilocarpine-stimulated Saliva of Normally Caries-resistant Animals Compared with Caries-resistant and -susceptible Humans

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In a recent study of the infrared spectral characteristics of human whole stimulated saliva collected from individuals classified as to dental caries status, a correlation was found between absorption peaks located at 7.2 and 12 μ, and the caries status of 398 airmen recently inducted into the U.S. Air Force.[1] Also there was direct correlation between flow rate and these infrared absorption peaks.

In order to reinforce such presumptive evidence that the characteristics of the infrared absorption curves might be an indicator of caries-susceptibility status, a series of infrared absorption studies of the saliva of normally caries-resistant rodents and other animals was completed and the results compared with the composite curves of the previous human caries-resistant and caries-susceptible groups. Included in the study were pilocarpine-stimulated salivary specimens of the rat, hamster, rabbit, cat, dog, and monkey.

All animals were preliminarily anesthetized with sodium pentothal, with the exception of the rabbits, which were tranquilized with promazine hydrochloride.* Each was then given an intramuscular injection of a 100 mg/100 ml solution of pilocarpine in the following amounts: rats (0.8 ml.), hamsters (1 ml.), rabbit (1 ml.), cat (1 ml.), dogs (2 ml.), and monkeys (2 ml.). After the first drop of saliva was collected as a result of the pilocarpine stimulation (usually after about 5 minutes), a 15-minute collection was made. The specimens were then centrifuged at 1500 rpm (5° C.) for 5 minutes, at which time a 1-ml. aliquot was taken, placed in a lyophilyzing tube with 4 ml. of distilled water, and 1 gm. of potassium bromide added. After complete dissolution of the KBr, the contents of the tube were shell-frozen in acetone and dry ice and lyophilized. One hundred and fifty milligrams of the homogeneously dispersed lyophilyzate were used to make a KBr pellet. The infrared scan was accomplished by use of an automatic recording, double-beam instrument, modified Baird Associates 400B, operating at a speed of 15 minutes per scan and with the starting absorption arbitrarily established at 65 per cent.

The absorption curves for all experimental animals within the same species were identical. There was also a general similarity between the absorption curves of the saliva among the various species, although several specific absorption peaks were evident (Fig. 1). In evaluating the absorption peaks located at 7.2 and 12 μ, excessive masking in some species was evident in the former area, negating any comparison with

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* Sparine, Wyeth Laboratories, Inc., Philadelphia.

human data. The absorption curve seen at 12 μ for all the species compared favorably with the human caries-resistant, but not with the human caries-susceptible, group. Since the animals selected are normally caries-resistant, the findings indirectly support the original hypothesis that at least the component responsible for the absorption peak located at 12 μ is related to a human caries-resistant status.

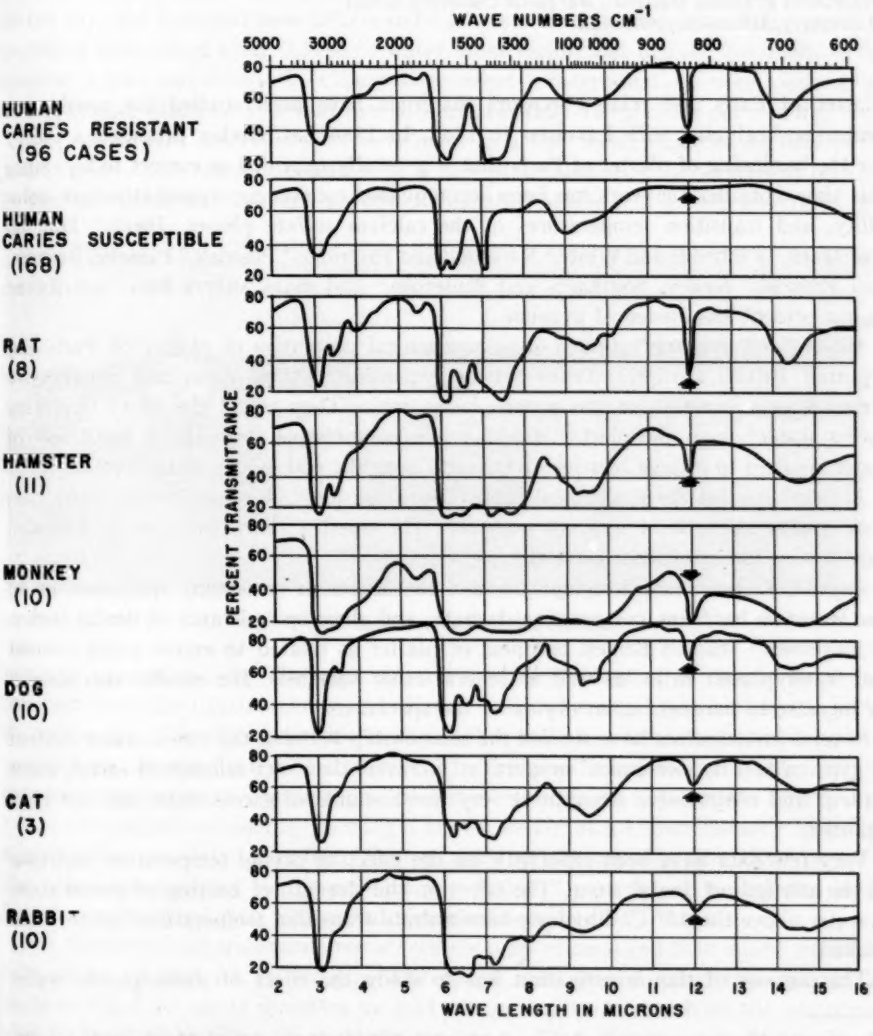

FIG. 1.—Infrared absorption curves of stimulated saliva of various species compared with that of caries-resistant and caries-susceptible humans.

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Compressive Properties of Dental Gypsum

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Plaster of Paris and related gypsum materials have been studied for nearly two centuries, beginning with Lavoisier in 1765. In 1884 LaChatelier proposed a theory for the hardening of plaster of Paris that is generally regarded as correct today. Since this time, considerable work has been accomplished concerning crystal structure, solubility, and transition temperatures of the calcium sulfate phases. Davis,[1] Dundon and Mack,[2] Partridge and White,[3] Ramsdell and Partridge,[4] Posnjak,[5] Palache, Berman, and Frondel,[6] Kelley, Southard, and Anderson,[7] and many others have contributed to our present knowledge of gypsum.

Gibson and Johnson[8] studied some mechanical properties of plaster of Paris and reported Brinell hardness, linear setting expansion, setting time, and compressive strength as a function of the water/plaster ratio. They noted the effect of drying excess water* from the plaster specimens on compressive strength. A small loss of water resulted in a large increase in strength near the end of the drying cycle.

Mahler[9] studied the mechanical properties of hardness, flow (by penetration), and compressive strength of gypsum materials. He noted a sharp increase in Rockwell hardness as his specimens lost water.

Peyton, Leibold, and Ridgley[10] studied the effects of immersing specimens in oil and water on hardness, compressive strength, and abrasion resistance of dental stones.

Jørgensen[11] studied Brinell hardness of plaster as related to excess water content and water/plaster ratio, as well as several other variables. His results also showed an increase in hardness upon drying of the specimens.

Several investigators have studied the relationship between the excess water content of gypsum and its mechanical properties; however, the exact relation of excess water content and compressive strength at very low amounts of excess water has not been reported.

Very few data have been reported[12] on the effect of drying temperature and time on the strength of dental stone. The effect on the strength of heating of dental stone in water above the 98° C. dihydrate-hemihydrate transition temperature has not been studied.

The purpose of this investigation was to study the effect of water/powder ratio,

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Portions of this paper were submitted to the faculty of the Graduate School, Marquette University, in partial fulfilment of the requirements for the degree of Master of Science.

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* The term "excess water" is used to designate the free water or water not used in the hydration to form gypsum.

excess water content, temperature during the storage in air, storage in water, gypsum solution, oil, and 100 per cent ethyl alcohol on the compressive strength and plastic deformation (flow) in compression of dental stones.

MATERIALS AND METHODS

Three dental stones—two hydrocal type (brands A and B) and one densite type (brand D), and hydrocal base B* (brand C)—were used in the study. The hydrocal specimens were mixed using 0.24–0.36 water/stone ratios. The densite specimens were mixed at a 0.24 and 0.22 ratio. Compressive-strength determinations were made on a Riehle Testing Machine at a load rate of approximately 700 pounds per minute.

Compressive-flow determinations were made in a flow meter designed for this purpose (Fig. 1). This machine consisted of a base plate fastened to a permanent bench. To the base plate was fastened an upright member which held a long lever arm by

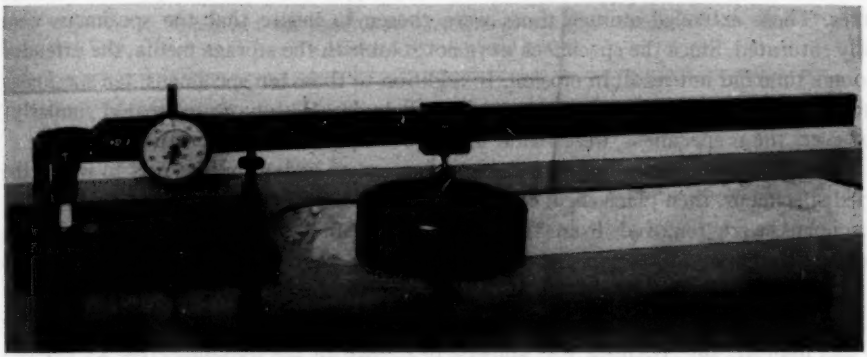

FIG. 1.—Compressive flow testing machine

means of a machined pin. The lever arm was capable of pivoting on this fulcrum pin. One inch from the fulcrum center, a universal ball-socket anvil was held to the lever by another pin. Directly under this anvil, another anvil was located on the base plate to which was fastened a plastic container. The container was filled with water during tests for wet specimens. Specimens to be tested were placed between the anvils, and a load was applied to them by hanging a known weight at a known distance from the fulcrum. The calculated maximum load capacity of the machine was 2,000 pounds. The calculated accuracy of the machine was within 5 per cent of the selected load. Measurements were taken with a dial gauge calibrated in 0.01 mm.

The distilled water and stone were accurately proportioned and then mixed mechanically under vacuum for 30 seconds. The mixed stone was vibrated into cylindrical lucite molds, 1.30 cm. in diameter by 2.54 cm. in length. After 1 hour the specimens were surfaced while still in the molds by sanding on 400A Aloxite paper. All specimens were dried at laboratory temperature and humidity for more than 2 days, then placed in a desiccator over calcium chloride for 48 hours. Specimens to be tested dry were used directly from the desiccator, and those to be tested wet were placed in distilled water for 30 minutes and then tested. The drying and wetting times indicated were

* United States Gypsum Company.

found to be sufficient for complete drying or wetting, as evidenced by serial weighing experiments. Additional wetting or alternate wetting and drying resulted in a larger experimental error and a slight lowering of mean strength values. These findings were established in a pilot study designed to develop the experimental procedure.

EXPERIMENTAL METHODS

The average compressive-strength values were obtained for ten specimens for wet condition and ten specimens for dry condition, for each water/powder ratio. In order to study the effect on compressive strength of wetting dental stone with liquids which cause little or no solution, ten specimens of one hydrocal stone of 0.28 water/powder ratio were dried as previously described, and five were immersed in transformer oil and five in 100 per cent ethyl alcohol. The specimens were allowed to remain in the alcohol for 4 hours prior to testing for compressive strength. The specimens immersed in oil were allowed to remain in the oil for 24 hours, and vacuum was applied at various times. These extended storage times were chosen to insure that the specimens were fully saturated. Since the specimens were not soluble in the storage media, the extended storage time did not result in erosion. In addition to these ten specimens, ten specimens each of the second hydrocal stone and base B hydrocal stone were treated similarly; however, these specimens were made using several water/powder ratios.

The method for controlling and determining excess water content was to weigh the dried specimens, then place them on an aluminum screen in a humidifier to gain water. Specimens were removed from the humidifier at predetermined intervals of time, weighed, and tested for compressive strength. Water was also added to some specimens in small quantities by a capillary tube, in order to obtain larger quantities of water in these specimens than they would gain from the humidifier. When water was added in this manner, the specimen was allowed to remain in the humidifier for 15 minutes to allow apparent dispersion of the water into the specimen before weighing and testing. Hydrocal stone specimens of 0.28 and 0.36 and densite stone specimens of 0.24 water/powder ratio were used for these determinations. A total of 63 specimens was tested in this manner.

The effect of temperature of storage in air at 65°, 75°, 85°, and 100° C. was studied by testing specimens for compressive strength after certain intervals of storage in an electric oven. In each instance the specimen was tested immediately after its removal from the oven. Each specimen was inserted in a foam-rubber collar to minimize heat loss during the test. The cylindrical holes in the foam-rubber collars were of the same diameter as those of the specimens. Therefore, the collars did not exert confining pressures. The weight loss of specimens at the end of each drying period was determined by weighing each specimen after it had cooled in a desiccator. X-ray diffraction recordings were taken of samples treated in a similar manner. The X-ray data were used to determine changes in the relative quantities of dihydrate and hemihydrate.

In an attempt to determine whether a strength loss occurred after storage near or above the hemihydrate-dihydrate transition temperature, several series of specimens were stored in gypsum solution, water, and water vapor at 100° C. for periods of time up to 24 hours. Specimens were removed at various intervals and tested for compressive strength. These specimens were also inserted in heated foam-rubber collars during the test period.

Stone specimens of 0.30 water/powder ratio were tested in the flow machine in a wet condition and in a dry condition with various test loads. The data reported and used to construct the flow curves are for the test loads of 2,450 and 3,900 psi for the wet specimens and 8,750 psi for the dry specimens. The loads of 3,900 and 8,750 for wet and dry specimens were selected to establish a load of approximately 80 per cent of the mean compressive strength for these two types of specimens. Since the 80 per cent strength load resulted in fracture of the wet specimens at an early time, it was decided to include experiments using a load of 2,450, which corresponds to approximately 50 per cent of the compressive strength, thereby making it possible to extend the observation time before fracture. In addition to these tests, several specimens were tested by increasing the load by a constant amount at 1-minute intervals, at which time a reading of the dial gauge was obtained.

TABLE 1*

MEAN COMPRESSIVE STRENGTHS OF DENTAL STONES IN POUNDS PER SQUARE INCH

Ratio	Wet Strength						Dry Strength					
	Brand A		Brand B		Brand C		Brand A		Brand B		Brand C	
	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ
36....	3,300	4	3,000	5	3,300	8	8,200	7	6,500	6	7,600	5
34....	4,000	3	3,100	8	3,700	8	8,500	8	6,500	7	7,900	4
32....	4,100	4	3,700	8	4,100	4	9,500	7	7,700	4	8,800	7
30....	4,700	3	4,100	5	4,100	9	10,900	5	8,900	8	10,400	8
28....	5,500	2	4,700	6	5,200	8	12,100	3	10,500	4	10,700	13
26....	6,000	3	5,100	3	5,300	5	14,600	6	10,600	7	12,900	6
24....	6,900	3	5,400	6	5,700	5	15,900	17	12,900	9	13,200	13

* $\bar{X}$ = mean compressive strength in psi; σ = relative standard deviation in per cent of mean strength of ten specimens.

In order to observe the wetting and drying of gypsum dihydrate particles, dilute solutions of chemically pure dihydrate, as well as solutions from dental stone specimens, were placed on a glass slide. These samples were observed under a microscope as they dried and again upon wetting with water. A filar eyepiece was used to estimate the particle sizes.

RESULTS

The mean compressive strengths and relative standard deviations in per cent for the hydrocal dental stones are given in Table 1.

An increase in strength was found as the water/powder ratio decreased for both wet and dry conditions. In Figure 2 the results obtained for material *A* are shown in the form of a graph. Schiller[13] has shown that the water ratio is a logarithmic function of the strength of plaster. The values obtained in this study also generally exhibited such a relation. It should be noted that the significance of each strength value was determined by the standard deviation, and all values in Table 1 are rounded off to the nearest 100 psi.

Specimens which were tested after immersion in oil and others tested after immersion in 100 per cent ethyl alcohol exhibited mean strength values of the same order as did similar specimens tested in the dry condition. Some of the values are given in Table 2. Similar values were obtained for the other stones at different water/powder ratios.

The differences between dry strength and strength after immersion in oil or alcohol were of the order of 3–5 per cent, and these differences were not statistically significant. The strength values for specimens immersed in water are given to illustrate the difference in wet and dry strength for such specimens.

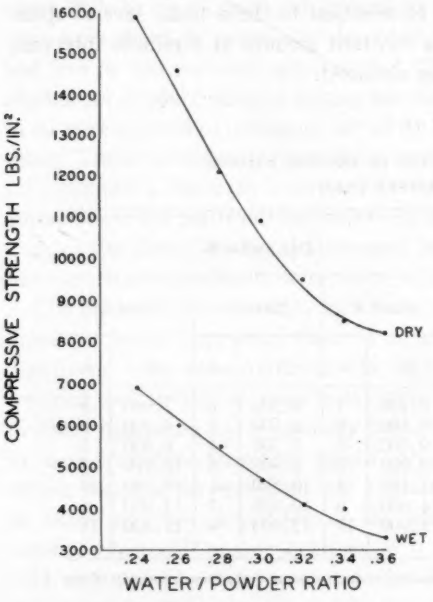

FIG. 2.—Compressive strength of material *A* plotted against the water ratio for wet and dry specimens.

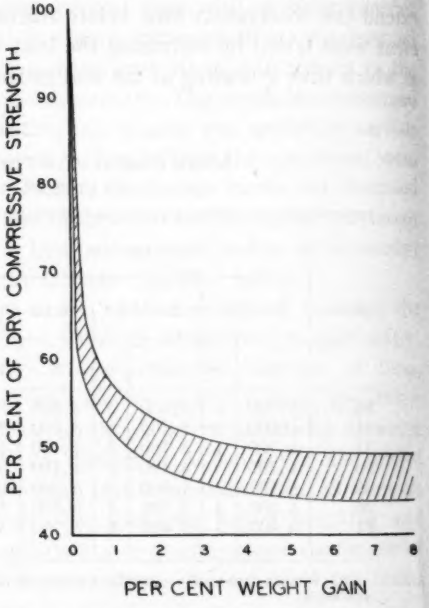

FIG. 3.—The per cent of dry strength of specimens plotted against the per cent excess water contained in the specimen. Materials *A* (W/P 0.28 and 0.36), *B* (W/P 0.28), *C* (W/P 0.28), and *D* (W/P 0.24) were used.

TABLE 2*

MEAN COMPRESSIVE STRENGTH OF BRAND A DENTAL STONE 0.28 W/P RATIO, TESTED DRY AND SATURATED WITH OIL, ALCOHOL, AND WATER

DRY		OIL		ALCOHOL		WATER	
$\bar{X}$*	σ	$\bar{X}'$	σ	$\bar{X}'$	σ	$\bar{X}$*	σ
12,100	3	12,400	5	11,500	6	5,500	2

$\bar{X}$ = mean strength of ten samples in psi; $\bar{X}'$ = mean strength of five samples in psi; σ = relative standard deviation in per cent of mean strength.

The compressive strength and the excess water content at the time of testing are given in Table 3 and in graphic form in Figure 3.

It was found that small weight increases, due to water gained, caused a large reduction in strength from the mean dry strength of the material tested. The five groups of specimens—stone A of 0.28 and 0.36 water/powder ratio, stones B and C of 0.28 water/powder ratio, and the densite stone of 0.24 water/powder ratio—exhibited similar reductions. The crosshatched curve in Figure 3 represents the general characteristics exhibited by the experimental values given in Table 3. In all instances the average wet-strength percentage level was reached between 1 and 2 per cent weight increase. Four of the five groups also showed about 25 per cent strength loss at 0.2 per cent weight increase. The fifth group lost 18 per cent strength at 0.2 per cent weight increase. It should be noted that 0.2 per cent weight increase was a very small quantity of water, from 11 to 13 mg. per specimen, depending on the group, and that the specimens did not appear to be wet. However, it was found that these and all other specimens which contained excess water broke during testing in a different manner than did dry specimens. The dry specimen shown in Figure 4, *a*, broke in a manner typical of all dry specimens. Specimens immersed in alcohol (Fig. 4, *b*) or in oil fractured in a manner similar to the dry specimens. The rupture occurred along a

TABLE 3

COMPRESSIVE STRENGTHS OF DENTAL STONES AS FUNCTION OF EXCESS WATER CONTENT

Stone A 0.28 Water/Powder		Stone A 0.36 Water/Powder		Stone B 0.28 Water/Powder		Stone C 0.28 Water/Powder		Stone D 0.24 Water/Powder	
Per Cent Water Gained	Per Cent Dry Strength	Per Cent Water Gained	Per Cent Dry Strength	Per Cent Water Gained	Per Cent Dry Strength	Per Cent Water Gained	Per Cent Dry Strength	Per Cent Water Gained	Per Cent Dry Strength
0.0	100*	0.0	100†	0.0	100‡	0.0	100§	0.0	100‖
0.1	90	0.1	74	0.2	77	0.1	84	0.1	68
0.1	86	0.1	70	0.2	68	0.1	89	0.2	72
0.1	81	0.1	76	0.4	61	0.2	82	0.8	58
0.2	74	0.2	76	0.4	60	0.4	82	0.8	60
0.2	74	0.2	69	0.5	61	0.4	66	1.2	56
0.3	59	0.7	56	0.6	56	0.4	60	2.4	52
0.9	57	0.8	55	0.6	55	0.7	56	4.2	46
0.4	56	1.2	46	1.3	51	1.0	57		
0.9	54	1.4	42	2.0	48	2.1	48		
0.6	54	1.6	51	2.2	46	5.4	50		
2.1	52	1.8	50						
6.2	50	1.9	53						
2.4	48	5.4	44						
2.7	47	6.6	47						
1.8	46	7.1	49						
3.8	45	7.5	44						
7.4	44	7.6	46						

* Dry strength, 12,100 psi. Wet strength, 5,500 psi. Wet strength in per cent of dry strength, 5,500/12,100 × 100 = 45 per cent.

† Dry strength, 8,200 psi. Wet strength, 3,300 psi. Wet strength in per cent of dry strength, 3,300/8,200 × 100 = 40 per cent.

‡ Dry strength, 10,500 psi. Wet strength, 4,700 psi. Wet strength in per cent of dry strength, 4,700/10,500 × 100 = 45 per cent.

§ Dry strength, 10,700 psi. Wet strength, 5,200 psi. Wet strength in per cent of dry strength, 5,200/10,700 × 100 = 49 per cent.

‖ Dry strength, 14,800 psi. Wet strength, 6,600 psi. Wet strength in per cent of dry strength, 6,600/14,800 × 100 = 45 per cent.

plane parallel to the direction of applied force, whereas the wet specimens (Fig. 4, *c* and *d*), ruptured along a plane at approximately 30° to the applied force. A similar pattern of rupture was observed for wet specimens tested for plastic deformation (flow) in compression.

The representative data for the plastic deformation of hydrocal stone 0.30 water/powder ratio are given in Table 4 and shown in graphic form in Figure 5.

In Figure 5, curves *a* and *b* represent the flow values obtained under 83 per cent load and 51 per cent load for the wet specimens. Curve *c* represents the flow values

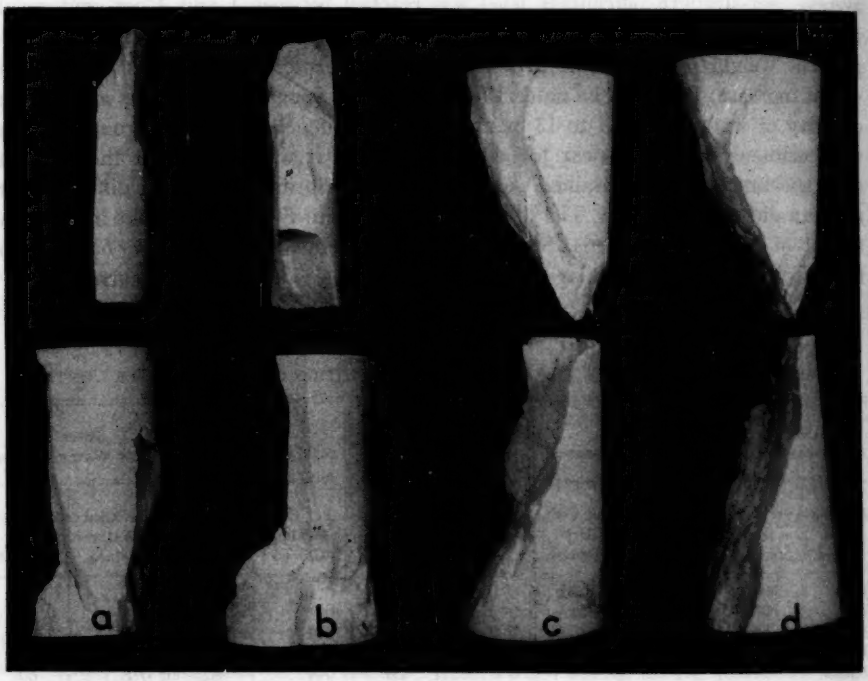

FIG. 4.—Broken test specimens. Specimen *a* dry when tested; specimen *b* wet with alcohol; specimens *c* and *d* wet with water.

obtained under 80 per cent load for the dry specimen. The small graph inserted at the right is constructed to show the form of the two curves *a* and *b* at the early stages of the test. When the two sets of curves are compared, it is found that curves *a* and *b* are similar in general characteristics. Each curve shows a linear portion and an increase in flow rate near the ends. A high initial flow rate was observed in all specimens tested, including the dry specimens, and this was considered to be caused by compression of small particles on the specimen ends.

The flow rates of the wet specimens increased with increase in the applied force, and the total plastic deformation before rupture was inversely related to the applied load. All wet specimens ruptured under the static loads applied, although these loads were considerably lower than the compressive strength. However, it should be noted that the time required to bring about rupture was increased from approximately 2

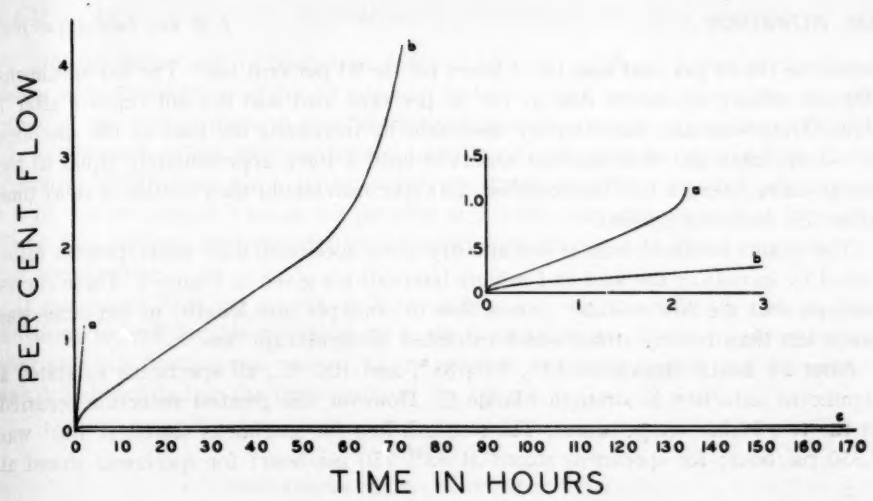

FIG. 5.—Per cent flow as a function of time for dental stone. Curve *a* represents values obtained when 83 per cent of wet strength was applied to the specimen. Curve *b* represents similar values when 51 per cent load was applied to the specimen. The small insert illustrates the early time portions of the curves. This curve also represents per cent flow as a function of time in hours.

TABLE 4

PER CENT PLASTIC DEFORMATION IN COMPRESSION OF DENTAL STONE
0.30 WATER/POWDER RATIO

HOURS: MINUTES	LOAD		
	3,900 psi 83 Per Cent Wet	2,450 psi 51 Per Cent Wet	8,750 psi 80 Per Cent Dry
0:03	0.07	0.03	.....
0:06	0.10	0.05	0.01
0:09	0.12	.....	.....
0:12	0.14	.....	.....
0:15	0.16	0.08	0.02
0:30	0.26	0.11	.....
0:45	0.34	.....	.....
1:00	0.42	0.14	0.03
1:30	0.70	.....	.....
2:00	0.89	0.21	.....
2:05	0.94	.....	.....
2:10	1.05	.....	.....
2:11	1.13*	.....	.....
3:00	.....	0.27	.....
4:00	.....	0.31	.....
8:00	.....	.....	0.04
12:00	.....	.....	.....
24:00	.....	0.98	0.05
48:00	.....	1.76	0.05
70:00	.....	3.58	.....
72:00	.....	4.16*	0.05
168:00	.....	.....	0.06

* Rupture occurred shortly after this reading.

hours for the 83 per cent load to 72 hours for the 51 per cent load. The dry specimens did not exhibit significant flow at the 80 per cent load and did not rupture after 7 days. Tests were also made on dry specimens by increasing the load on the specimen at 1-hour intervals; flow was not observed until a force approximately equal to the compressive strength had been applied. The specimen would then rupture a short time after this load was applied.

The results obtained from a wet and dry stone specimen, 0.30 water/powder ratio, tested by increasing the load at 1-minute intervals are given in Figure 6. These curves indicate that the flow modulus (stress/flow in units per unit length) of wet stone was much less than for dry stone, which exhibited no significant flow.

After 24 hours' storage at 65°, 75°, 85°, and 100° C., all specimens exhibited a significant reduction in strength (Table 5). However, the greatest reduction occurred at the two higher temperatures. The strength loss for specimens stored at 100° was 2,350 psi/hour; for specimens stored at

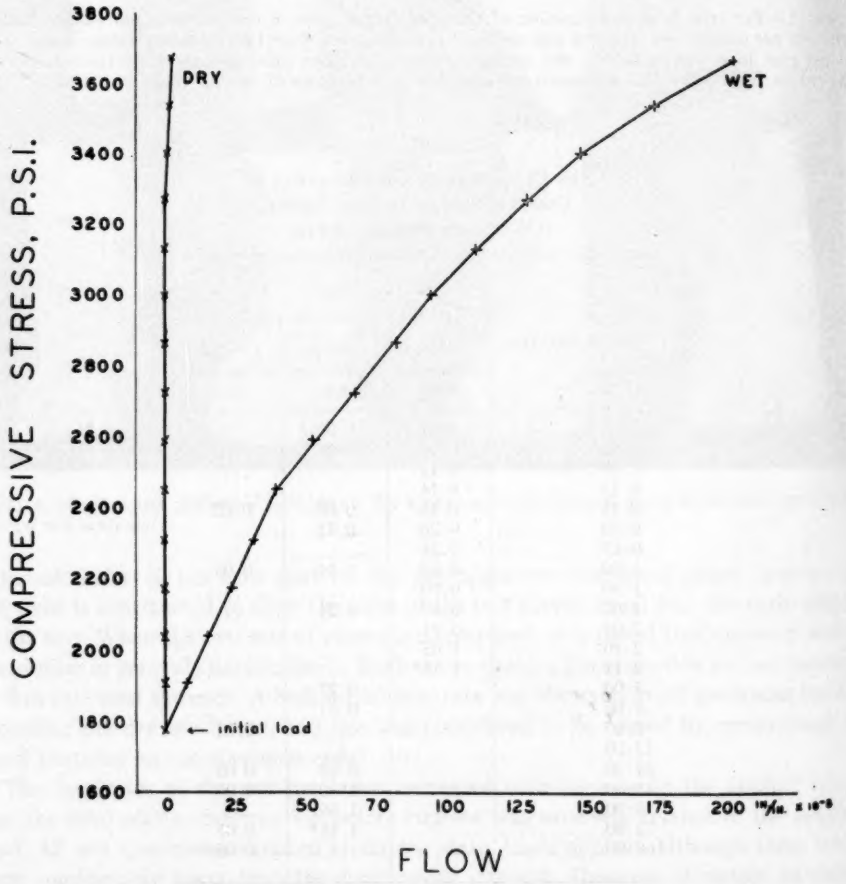

FIG. 6.—Stress-flow curves for wet and dry stone water/powder ratio 0.30, tested by increasing load at 1-minute intervals.

75°, 200 psi/hour; and for specimens stored at 65°, 150 psi/hour. Although these rate values were obtained for different intervals of the 24-hour period, they gave an indication of the rate of strength loss before the minimum strength values were reached. These minimum values were considered to be due to the mechanical structure of the specimen. Weight loss for a typical specimen stored at 85° C. was 0.8 per cent at 1 hour, 1.6 per cent at 3 hours, 6.8 per cent at 9 hours, and 14.1 per cent at 24 hours. These data indicate that dehydration occurred concurrently with the strength loss at this temperature.

It was found from the results of X-ray diffraction recordings that a change from dihydrate to hemihydrate had occurred in the heated samples. X-ray recordings taken after alternate heating and removal of the exposed surface indicated that the surface was dehydrated before the inner parts of the sample.

TABLE 5

COMPRESSIVE STRENGTH IN PSI OF DENTAL STONE AFTER STORAGE AT 65°, 75°, 85°, AND 100° C.

TIME (HOURS)	TEMPERATURE (° C.)			
	65	75	85	100
2	.....	.....	.....	11,000
3	.....	.....	13,000	7,000
4	.....	.....	.....	6,000
5	.....	.....	.....	4,000
6	11,500	11,000	10,500	.....
8	.....	.....	8,500	.....
10	11,000	10,500	.....	.....
12	.....	.....	5,500	.....
24	9,000	7,500	3,000	3,000

It was noted that specimens stored in water vapor at 100° C. for 6–24 hours gave rather uniform strength values corresponding to approximately 90 per cent of the mean wet-strength value, whereas the strength of specimens stored at 100° C. in gypsum solution or in water for 6–24 hours varied from 50 to 90 per cent of the mean wet-strength value. Such large variations in strength of these specimens is thought to be due to sample erosion resulting from action of the storage medium.

Microscopic examinations of gypsum crystals on a glass slide revealed the particle size to range from less than 1 to 10 μ. Observations of the gypsum crystals during wetting and drying cycles revealed that the water entered and left these crystals through the narrowest interstices.

DISCUSSION

The increase in compressive strength due to loss of excess water demonstrated in this study corresponds to the increase in hardness found by Mahler[9] and Jørgensen.[11] The dry-strength results of this study, however, are higher than might be expected if the specimens had been stored in laboratory air and humidity. The results of strength loss at various temperatures are of significance in relation to some of the

current technics of drying stone investments and models. It was found that at 85° C., dehydration of the surface and strength loss occurred very rapidly. The evidence obtained by means of X-ray diffraction recordings and by weight-loss determinations on specimens upon heating substantiate the fact that dihydrate is transformed into hemihydrate.

The results obtained for the compressive flow of dental stone indicate that wetting of the stone permits plastic deformation to occur in the specimen. The marked similarity in the curves obtained for the flow of stone and high-temperature flow (creep) of metals leads one to believe that an analogous mechanism may be responsible for this similarity. The flow of wet dental stone would seem to result from the breakdown of the material between large crystals of gypsum. In metals, the mechanism is more complex, but the presence of grain boundaries has considerable influence on creep properties. It should be noted that the sharp cleavage plane resulting from rupture of a dry specimen indicates that rupture does not occur because of large plastic flow between crystals but because of disruption of the crystals themselves.

Further consideration of the data obtained indicates a marked difference in relative flow moduli of the dry and wet stones. This suggests that a structural change occurs in the polycrystalline gypsum specimen as it changes from the wet to the dry condition. This is further indicated by the different fracture characteristics of the dry and wet specimens.

X-ray diffraction studies of gypsum in a wet and a dry condition indicate that no change of measurable magnitude occurs in the interplanar spacings. Therefore, it is believed that the structural change occurs at the intercrystalline boundaries. These boundary areas are the shortest distances between the crystals and, consequently, the last locations to lose water during drying and the first locations to receive water during wetting of the gypsum. A continuous precipitation of gypsum occurs in the gypsum specimen because of the tendency toward an increase in gypsum concentration during evaporation of water. At some critically low water content, the majority of the gypsum-water solution is located only in the smaller crystal boundary areas. Since the space available for the precipitated crystals is small, an intercrystalline union forms and continues to build until all water is evaporated. On entry of the water, these same areas are first attacked by the water, and the union is dissolved and the strength lowered (Fig. 7, *A–F*).

After approximately a 2 per cent gain of water, no further loss of strength occurs. This indicates that the wet strength is a result of the mechanical structure of the specimen and that it is dependent upon the presence of the large gypsum crystals.

When dry specimens are immersed in alcohol, it is unlikely that the intercrystalline union is dissolved. The low solubility of gypsum in alcohol would then account for the similarity of strengths of dry specimens and of specimens immersed in alcohol. The hypothesis that the dry strength of gypsum is due to the mechanical interference of the small secondary crystals deposited on the larger crystals during drying is supported by this evidence.

The formation of these small secondary crystals and their strength contribution can be demonstrated by placing two samples end to end, after briefly immersing them in water, with a 500-gm. weight applied while the specimens are dried completely. Upon removal of the weight after complete drying, the specimens will be firmly at-

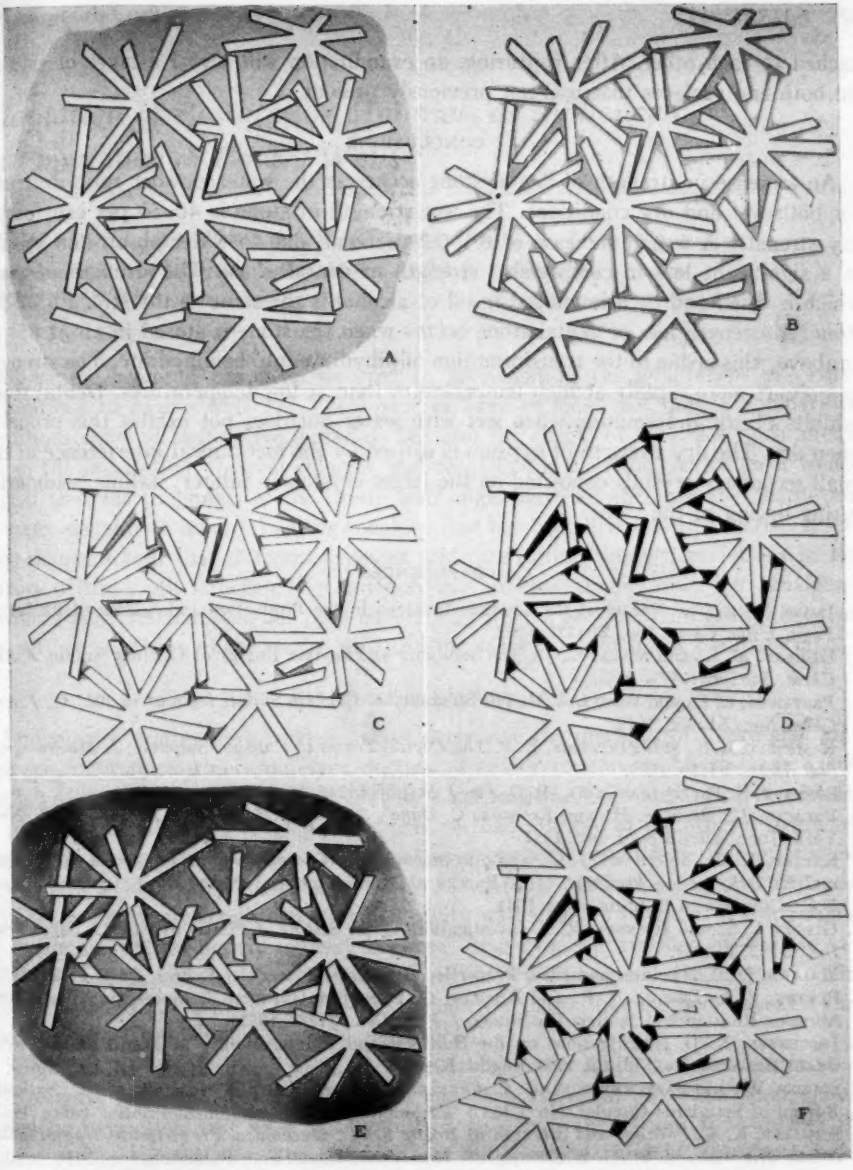

FIG. 7.—*A*, drawing of a field in a wet polycrystalline gypsum specimen. Only a few crystal projections are shown originating from a common center. *B*, First stage of drying. Each crystal is completely surrounded by gypsum solution, but the large voids between crystal projections have emptied. Precipitation on all faces of crystals occurs at this stage but is not illustrated. *C*, Second stage. Remaining gypsum solution is located at the intercrystalline boundaries. Precipitation occurs in these limited areas. *D*, Dry gypsum. All water has evaporated, and precipitation is completed with the formation of a solid union at the intercrystalline boundaries. *E*, Wet gypsum specimen under compressive force. The gypsum crystals can move (flow) with respect to one another. Under sufficient force the crystals can slide past each other with only an occasional fracture of the crystal projections. *F*, Dry gypsum. Intercrystalline unions prevent flow. The rigid crystals fracture instead of slipping. Whether the fracture occurs at the newly formed union or at some other point in the parent crystal would make no difference. The fracture is essentially along a plane. The separating force may be a complex force (shear, tension, compression).

tached to each other. After rupturing, an examination will reveal a layer of crystals on both end surfaces that was not previously present.

CONCLUSIONS

An increase in strength of dental stone occurs as the water/powder ratio decreases for both wet and dry conditions. The wet strength of stone is 40–50 per cent of the dry strength. A weight increase of 0.1–0.2 per cent, due to water absorption, results in a significant loss in compressive strength of dental stone. The strength of stone which is dried and then immersed in oil or alcohol is the same as the strength of dry stone. A strength loss of dental stone occurs when the stone is stored in air at 65° C. or above; this is due to the transformation of dihydrate into hemihydrate. The strength loss occurs more rapidly at high temperatures than at low temperatures. Dental stone exhibits plastic deformation when wet with water but does not exhibit this property when dry. The dry strength of gypsum is caused by the mechanical interference of the small secondary crystals deposited on the larger crystals in intercrystalline boundaries during drying of the stone.

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Adaptation of Restorative Materials as Influenced by Roughness of Cavity Walls

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Considerable research has been devoted to investigating the adaptation of dental restorations to cavity walls.[1-17] These studies have included not only a comparison of various common restorative materials but also the effects of variables, such as the age of the restoration, thermal changes, and technics for insertion. Although it would appear that the roughness of the cavity wall might have an effect on the adaptation of many restorative materials, little consideration has been given to this factor. It has been demonstrated that different types of instrumentation produce variations in the texture of the tooth structure,[18-23] but only one investigation,[24] limited to amalgam- and gold-inlay restorations, has been concerned with the possible relationship between the surface condition of the cavity wall and the sealing properties of the material.

MATERIALS AND METHODS

Preliminary studies were carried out to determine the methods of instrumentation that would produce widely different degrees of cavity roughness. Microscopic examination of many specimens revealed that the Cavitron, an ultrasonic device, consistently produced cavity walls of a smoother texture than did any of the other instruments employed. A typical surface, on the labial of a cuspid, prepared by this instrument is shown in Figure 1. By comparison, a No. 558 carbide bur rotating at 250,000 rpm* resulted in extremely rough and irregular cavity-wall surfaces (Fig. 2). A third degree of roughness, approximately midway between these two, was attained by the use of a No. 558 carbide bur with conventional-speed rotary equipment (5,000 rpm). This surface appears in Figure 3. Repeated cuts made by these three methods revealed that these surfaces could be consistently reproduced.

Simple cavity preparations (Fig. 4) were made on the labial or buccal surfaces of 280 recently extracted teeth. These teeth had been stored in tap water immediately following extraction and were not permitted to dehydrate at any time during the course of the experiments. The size of the cavities was controlled, the depth being approximately 2.5 mm. and the diameter 2.2 mm. A water spray was used while cutting with the rotary instruments.

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* Borden Air Rotor.

Five different materials—amalgam, silicate,* an autopolymerizing resin,† cohesive gold foil, and mat gold foil—were placed in the cavities having the three different types of cavity walls. Accepted standardized procedures were used with all materials.

A 5/5 mercury-alloy ratio, as advocated by Eames,[25] was used for the amalgam restorations. The amalgam was triturated mechanically and condensed by a mechanical condenser. The restorations were polished 24 hours after insertion, with care being taken to avoid excess heat.

Definite powder-liquid ratios were established for both the silicate and the resin materials. The silicate cement was mixed on a cool slab for a maximum of 30 seconds. Pressure was applied with a celluloid strip for 3 minutes after insertion of the material. Upon removal of the strip cocoa butter was applied. Fifteen minutes later the excess was carefully removed with a fine-grit cuttle-fish disk and the restoration again coated with cocoa butter. The restorative resin was introduced into the cavity preparation by the flow technic.[26] The cavity liner supplied by the manufacturer was employed

* S. S. White. † Sevriton.

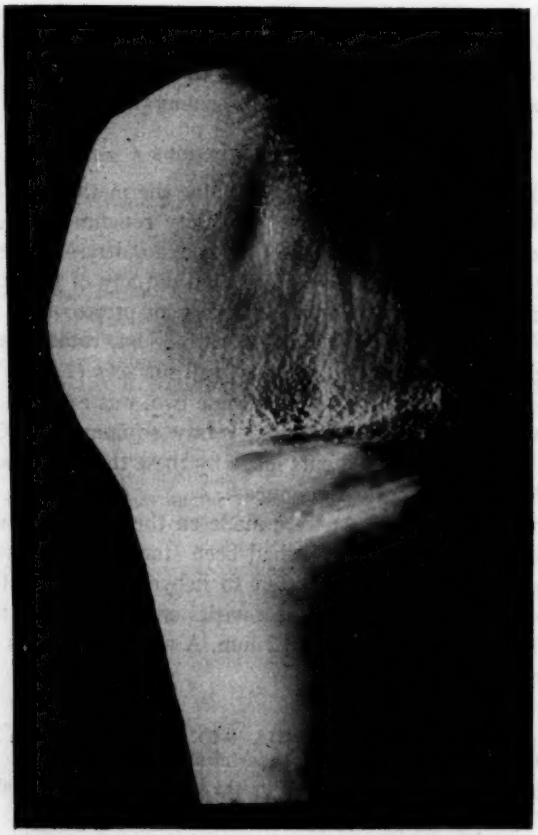

FIG. 1.—Labial surface of tooth cut by Cavitron (Orig. mag. ×10)

with this material, and final finish of the restoration was carried out 24 hours after insertion.

Both the cohesive and the non-cohesive mat gold were condensed with a magnetic condenser.*

Following placement of the restorations, the teeth were placed in water and stored at 37° C. Since there is evidence that the adaptation of certain materials is influenced by the age of the restoration,[7, 12, 13] tests for leakage were made at intervals of 48 hours, 30 days, and 90 days.

The relative adaptation of the restorations was determined by the amount of radioisotope that infiltrated at the margin between the cavity walls and the filling material during a given period of time. The exact test method, employing a $Ca^{45}Cl_2$ solution (0.1 mc/ml and adjusted to pH 5.5) has been described in a previous publication.[12] The teeth were sealed, except for the restoration and the area immediately surrounding it, with a combination of fingernail polish and tin foil. They were then placed in the radioactive solution for 2 hours. Upon removal from the isotope, the sealing material

* Electro-Mallet.

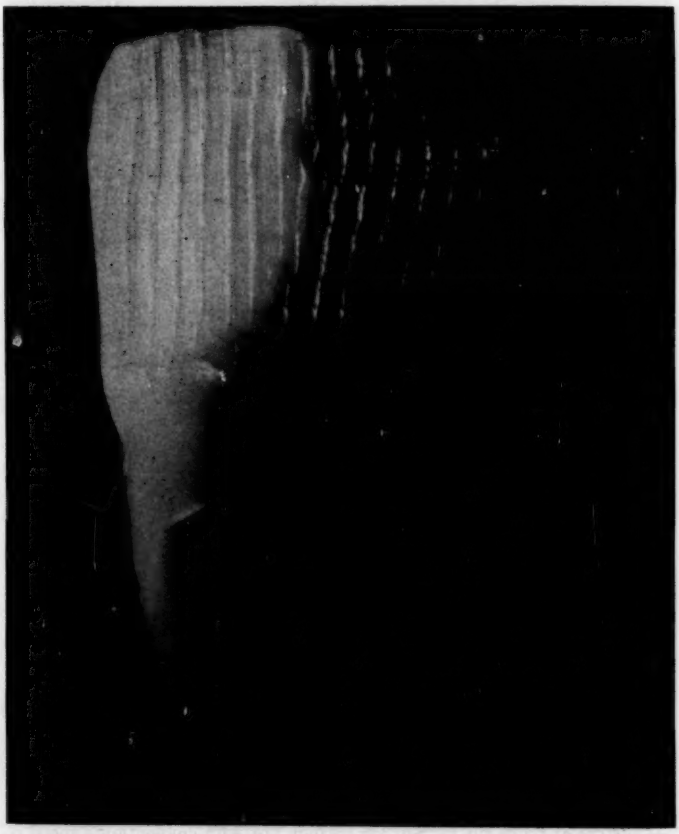

FIG. 2.—Labial surface of tooth cut by No. 558 carbide bur with Borden Air Rotor. (Orig. mag. ×10)

was stripped away, and the surfaces were carefully scrubbed with detergent and water. Longitudinal sections, parallel to the long axis of the tooth, were made through the restorations in order to expose the cavity margins. The teeth were allowed to remain in contact with the X-ray film for 24 hours, and the film was then processed. Particular care was taken to standardize the immersion time of the tooth in the isotope, the exposure time of the teeth to the X-ray film, and the processing of the film.

For the most part, previous tracer studies on adaptation have not been quantitative but have merely been comparisons based on visual observation of the autoradiographs. Although this method is undoubtedly quite satisfactory for demonstrating the phenomenon and determining general trends, a more exacting method of measuring leakage is desirable if such variables as surface roughness are to be studied. After much experimentation, the following method was devised.

Basically, the calculation of leakage consisted of actually measuring the area infiltrated by the isotope and establishing a ratio between that area and the "leakage potential." The arbitrary term "leakage potential" is assigned to the perimeter of the

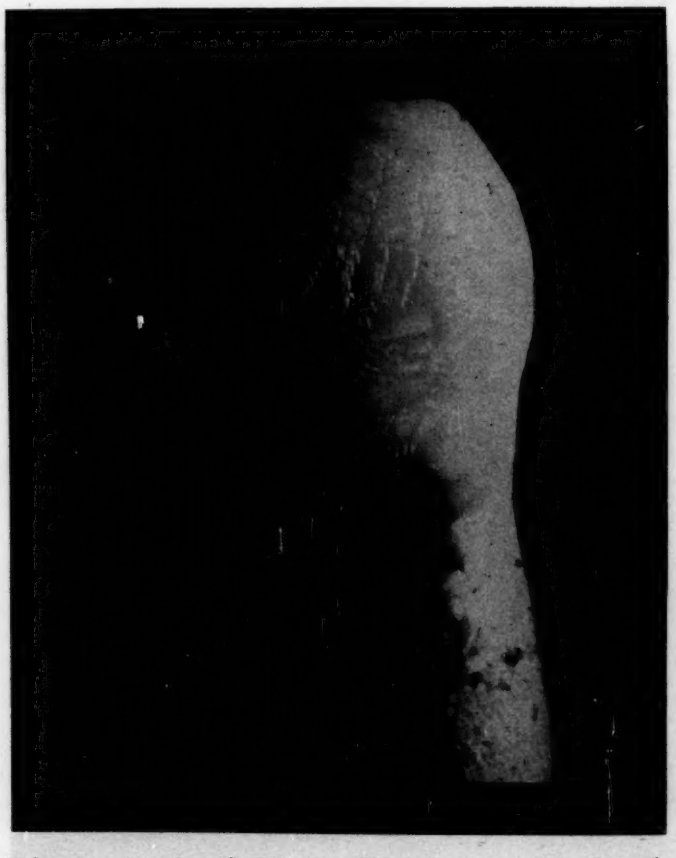

FIG. 3.—Labial surface of tooth cut by No. 558 carbide bur with conventional-speed rotary equipment. (Orig. mag. ×10.)

sectioned preparation (this being the total length around the cavity walls and the pulpal floor).

Measurement of the area infiltrated by the isotope was done by mounting the autoradiographs between glass and projecting the images onto pieces of drawing paper mounted on a vertical board. A microscope stage micrometer was modified for use on a slide projector, making it possible to adjust the distance between the projector and the drawing paper so that the autographs were enlarged exactly 50 times. With a very sharp pencil the areas of infiltration were outlined on the paper. The projected images revealed a sharp line of delineation around the leakage areas. Only the well-defined regions between the cavity walls and the floor were traced. Any fuzzy or indefinite shadows were ignored. Where the radioactive isotope had penetrated the dentinal tubules, these areas were not included, since dentin infiltration undoubtedly is related more to tooth permeability than to marginal leakage. In evaluating materials such as silicate, where the filling material itself was penetrated by the isotope, it was, of course, necessary to differentiate between this phenomenon and true marginal leakage. However, this could readily be done on the projected images.

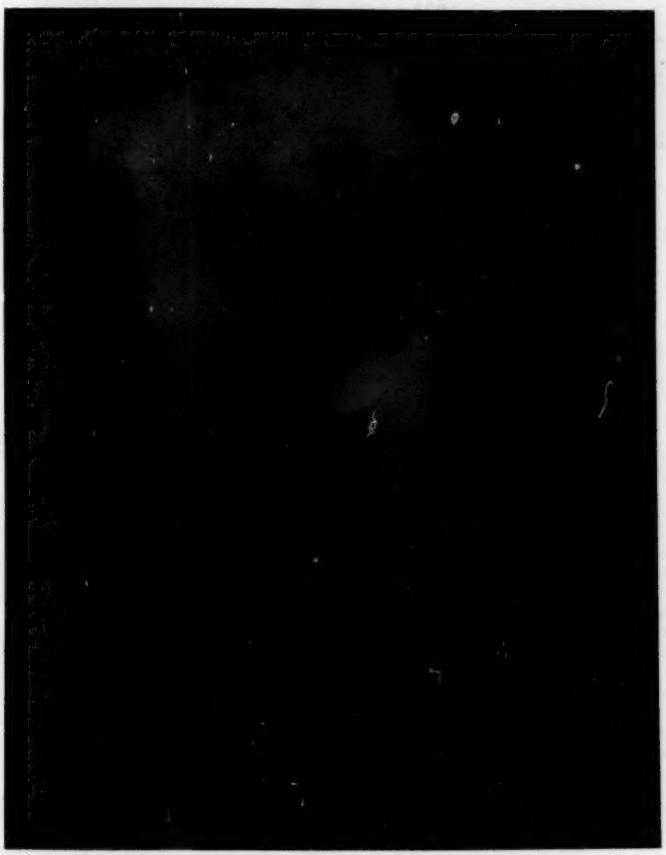

FIG. 4.—Type of cavity preparation employed

From these tracings, the area of leakage was determined by means of an instrument* designed for accurate measurement of plane areas of any form. To measure an area by the use of this instrument, it is only necessary to run the tracer point around the periphery of the figure and read the distance which the measuring wheel revolves during the process. The precision and accuracy of the instrument were determined with the aid of the testing rule supplied for this purpose. These tests showed the instrument to have a precision of 1:1000 when measuring a 10-square-inch area. The instrument was calibrated at 70° F., and all readings were made at 70° ± 2° F. A minimum of two measurements was made on each specimen.

A map-measuring device was used to determine the perimeter of the cavity preparation. This was done by measuring the cavity walls on the ground tooth specimen or, in those instances where the isotope had penetrated completely around the restora-

* Keuffel and Esser Compensating Polar Planimeter.

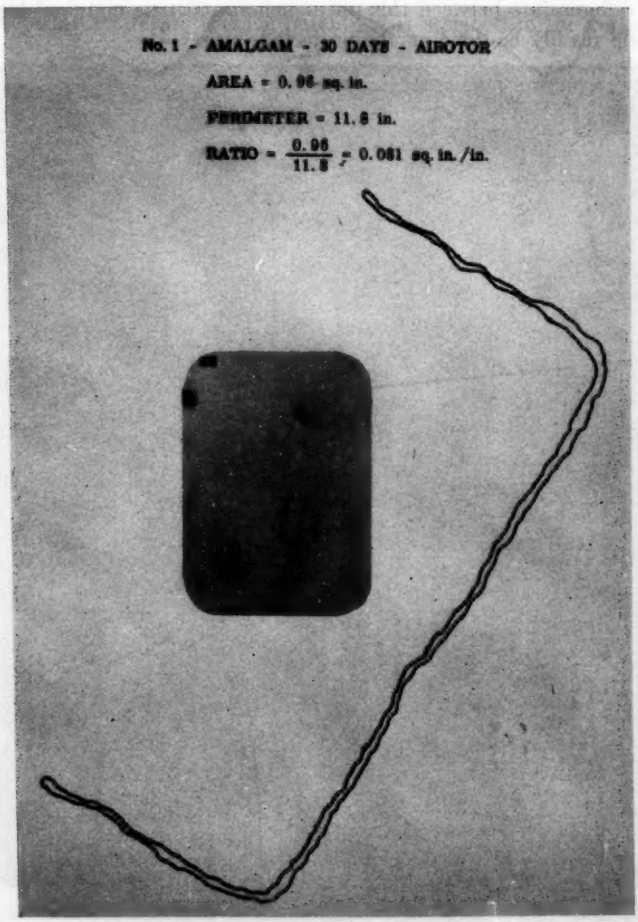

FIG. 5.—A typical autoradiograph, the tracing, and calculation of the leakage ratio

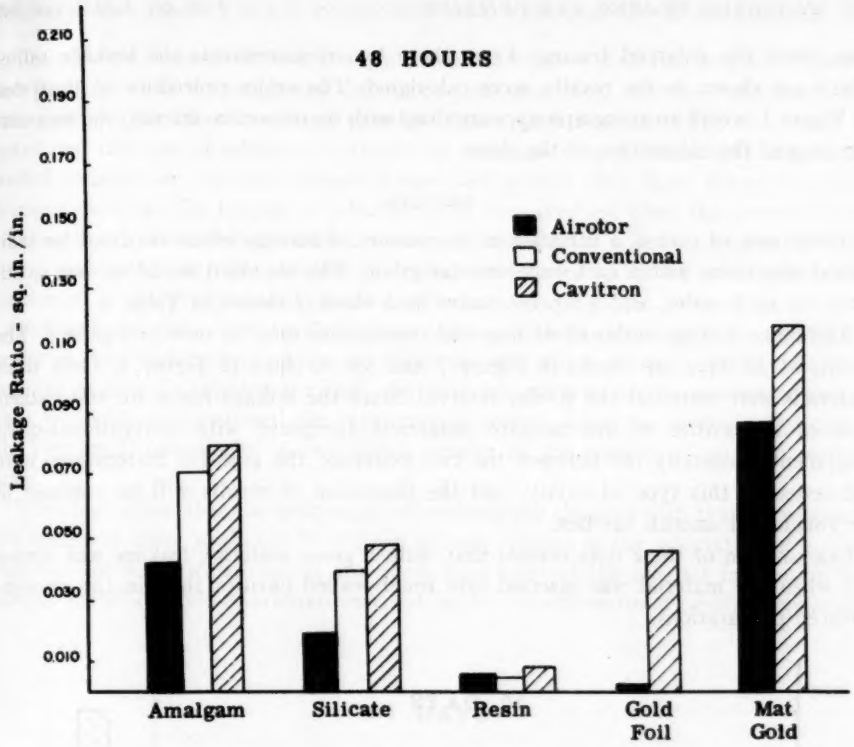

FIG. 6.—Mean leakage ratios on 48-hour-old restorations placed in cavities of three degrees of roughness.

TABLE 1

INDIVIDUAL RESULTS FOR AMALGAM RESTORATIONS PLACED IN THE THREE TYPES OF CAVITY PREPARATIONS AND TESTED 48 HOURS AFTER PLACEMENT*

AMALGAM—48 HOURS

Number of Specimen	Air Rotor			Conventional			Cavitron		
	A	P	R	A	P	R	A	P	R
1	0.34	9.0	0.037	0.57	9.1	0.062	1.06	10.7	0.099
2	0.34	9.4	0.036	0.72	9.4	0.076	0.65	9.0	0.072
3	0.38	9.5	0.040	0.51	8.7	0.057	0.75	9.4	0.079
4	0.35	8.0	0.043	0.98	9.9	0.098	1.05	10.8	0.097
5	0.48	9.1	0.052	0.67	9.3	0.072	0.59	8.2	0.071
6	0.29	8.1	0.035	0.64	8.8	0.072	0.67	8.9	0.075
7	0.30	8.0	0.037	0.73	10.1	0.072	0.65	8.9	0.073
8	0.50	9.3	0.053	0.81	9.8	0.079	0.72	9.9	0.072
9	0.46	8.2	0.056	0.60	9.2	0.065	0.62	8.1	0.076
10	0.24	8.7	0.027	0.58	9.4	0.061	0.76	10.5	0.072
Ratio average			0.042			0.071			0.079
Standard deviation			0.009			0.009			0.010

* The letters in the columns have the following meanings: A = area (sq. in.); P = perimeter (in.); R = ratio = A/P (sq in/in).

tion, from the enlarged tracing. From these two measurements the leakage ratios, which are shown in the results, were calculated. The entire procedure is illustrated in Figure 5, where an autograph appears along with its respective tracing, the measurements, and the calculation of the ratio.

RESULTS

There was, of course, a variation in the amount of leakage which occurred for individual specimens within each experimental group. The standard deviation was calculated for each series, and a representative data sheet is shown in Table 1.

The mean leakage ratios of 48-hour-old restorations may be seen in Figure 6. The results at 30 days are shown in Figure 7 and for 90 days in Figure 8. Only three materials were tested at the 90-day interval. Since the leakage ratios for restorations inserted in cavities of intermediate roughness (prepared with conventional-speed equipment) generally fell between the two extremes, the gold-foil restorations were not tested in this type of cavity, and the discussion of results will be confined to the rough and smooth cavities.

Examination of these data reveals that, with a given material, leakage was always less when the material was inserted into rough-walled cavities than in the smooth-surfaced preparations.

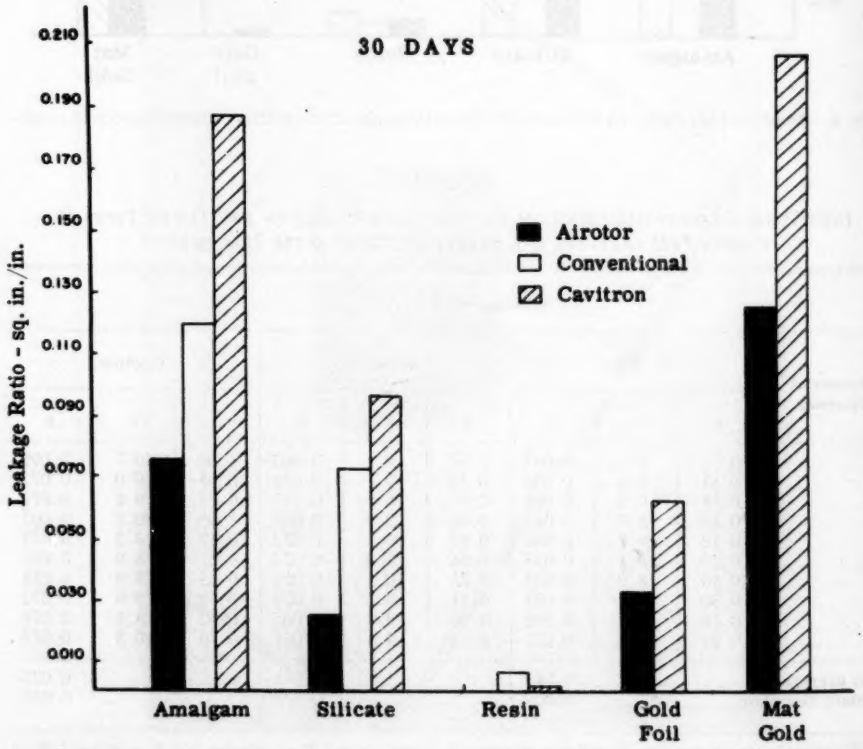

FIG. 7.—Mean leakage ratios on 30-day-old restorations placed in cavities of three degrees of roughness.

The differences observed with amalgam, silicate, cohesive gold foil, and non-cohesive mat gold placed in the rough and smooth cavities were most definite. Considering the data collected for the restorations tested 48 hours after insertion (Fig. 6), it will be noted that the area of infiltration around the amalgam restorations placed in smooth-walled preparations was approximately one-third greater than those placed in rough-textured cavities. The leakage of silicates more than doubled when the smooth cavity was employed. A marked increase in leakage occurred with cohesive gold-foil restorations, while a 25 per cent increase in leakage was noted with mat-gold restorations condensed in cavities prepared by the Cavitron.

Although the data consistently indicated the adaptation of resin to be slightly superior in the rough-walled cavity, the difference was slight. In fact, in only this series was the standard deviation for both the 48-hour and the 30-day specimens greater than the differences between the experimental groups. This was undoubtedly due to the very small quantity of isotope which penetrated the margins of all resin restorations.

The observation that the adaptation of restorations changes with time corroborates the results of other workers.[7, 12, 13] Even though changes in the adaptation of a given material did occur with time, at any given time interval the greatest infiltration was always found around restorations inserted in the smooth-textured cavity preparation.

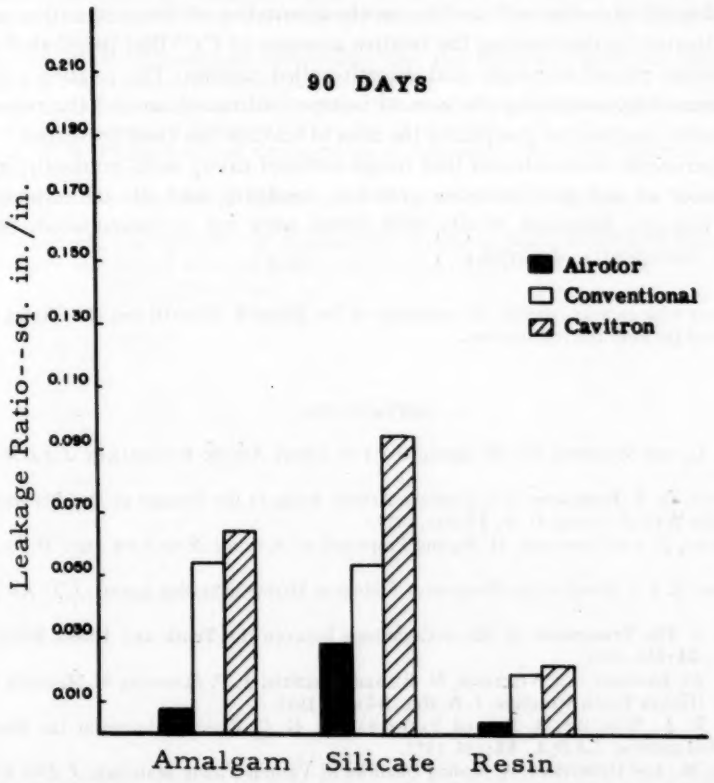

FIG. 8.—Mean leakage ratios on 90-day-old restorations placed in cavities of three degrees of roughness.

DISCUSSION

The results of this study indicate that the surface condition of the cavity wall is an important consideration in the complex problem of marginal leakage of dental restorations. In assessing the sealing properties *per se* of any restorative material, it is evident that the texture of the cavity preparation must be considered and controlled. Failure to control this variable may well be partially responsible for some of the divergent results obtained by different investigators.

Since all five restorative materials consistently exhibited better sealing properties when placed in a rough-textured cavity, it can only be assumed that the irregularities of the cavity walls provide for better retention of the restorative material.

Although there is no actual clinical evidence that leakage of the magnitude demonstrated in this investigation contributes to recurrent caries, considerable research effort is being expended to find means of securing better adaptation of dental restorations. These tests have shown that the adaptation of restorative materials can be improved by the use of cavity liners,[12, 13] altered manipulative technics,[10] and chemical treatment of the tooth surface.[27] The results of this investigation indicate that modification of the cavity wall by mechanical means should be added to this list.

SUMMARY

The influence of cavity-wall texture on the adaptation of five restorative materials was investigated by determining the relative amounts of Ca^{45} that penetrated margins of restorations placed in rough- and smooth-walled cavities. The relative adaptation was determined by measuring the area of isotope infiltration around the restorations. A quantitative method for computing the area of leakage has been presented.

The experiments demonstrated that rough-surfaced cavity walls markedly improved the resistance of mat-gold, cohesive gold-foil, amalgam, and silicate restorations to marginal leakage. Although results with resins were not so pronounced, the same trend was consistently observed.

The authors wish to acknowledge the assistance of Dr. James P. Vernetti and Dr. Charles Latham, who condensed the gold-foil restorations.

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A New Material and Technic in the Art of Somato-Prosthesis

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The search for materials to be employed in the fabrication of a somato-prosthesis dates back to the days of Pierre Fauchard (1541). In reporting this and other historical facts relative to this art, Kazanjian,[1] in an early work, describes the use of vulcanite rubber as a base material, the surface of which was covered with celluloid paints to match skin color. At a later date, Bulbulian[2] tells of his use of latex and methyl methacrylate as materials in prosthetic reconstruction of the congenital or postoperative loss of the ear. Seeking also to replace lost anatomical parts, Heron[3] and Bigelow[4] used acrylic resin to advantage, while Clarke[5] outlines his work with vulcanized latex in conjunction with oil-soluble dyes as coloring agents. In 1955 Ackerman[6] and Fonder[7] published work telling of the use of vinyl plastics and self-curing acrylic resin, respectively.

While the previously reported work dealt with both resilient and hard substances to achieve their results, the research herein recorded combines the physical and chemical features of both types.

MATERIALS AND METHODS

The following formula, proportionately increased or decreased as outlined in Table 1, and close adherence to all measurements and armamenta will immeasurably aid in circumventing many pitfalls: 24 ml. Silastic RTV 502;* 3 ml. 200 silicone fluid;† 4 ml. methyl methacrylate liquid resin;‡ 12 ml. methyl methacrylate powder;§ 125 mg. Silastic RTV 502 catalyst.||

The white base material is first colored to a pale tan by mixing 400 ml. Silastic RTV 502 with 8 ml. of a color paste consisting of 198.6 gm. of Silastic 502 and 1.4 gm. of Mapico Tan. This quantity will provide sufficient material for fabricating several somato-prosthetic appliances and nearly sufficient to produce all the colors in the tissue-color guide (Tables 2–5).

After determining the number of desired tissue colors needed for all specific areas

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* A fluid, quick-setting, room-temperature-vulcanizing silicone rubber. Dow Corning, Midland, Mich.

† Twenty centistokes. Dow Corning.

‡ Vitalon methyl methacrylate liquid resin. Austenal Dental Division, Chicago, Ill.

§ Vitalon methyl methacrylate color-form denture stain powder. Austenal Dental Division.

|| Stannous octoate. A carefully controlled test showed 25 mg. of the catalyst was equal to 1 drop from a Glasco Drop-a-Time straight medicine dropper and has been found to be the most practical means of measuring such small amounts of this material.

and the base color, the specific-area colors are introduced into the charted mold (Fig. 1).* It is suggested that one color formula at a time be mixed and placed in the desired area.

In a large glass syringe, the bottom of which has been cut away so as to allow the plunger, when fully seated, to be flush with the bottom of the syringe walls, the desired quantity of base material is accurately measured. It should be noted that the base material is rather thick, and care should be exercised not to entrap air, thus providing a false measurement. This material is then slowly expressed into a wax-lined paper cup (5-oz. size); excess pressure upon expressing could result in cracked syringe

* All armamenta referred to are pictured in Fig. 1.

TABLE 1

PROPORTIONS OF SILASTIC RTV 502, 200 SILICONE FLUID, LIQUID RESIN, AND COLOR-FORM DENTURE STAIN POWDER PLUS SILASTIC 502 CATALYST FOR SMALL, MEDIUM, AND LARGE MIX REQUIREMENTS

Small Mixes		Small Mixes	
4	ml. RTV 502	8	ml. RTV 502
0.5	ml. 200 fluid	1	ml. 200 fluid
0.7	ml. Vitalon liquid	1.4	ml. Vitalon liquid
2	ml. Vitalon powder	4	ml. Vitalon powder
25	mg. 502 catalyst	50	mg. 502 catalyst
Medium Mix		**Large Mix**	
16	ml. RTV 502	24	ml. RTV 502
2	ml. 200 fluid	3	ml. 200 fluid
2.6	ml. Vitalon liquid	4	ml. Vitalon liquid
8	ml. Vitalon powder	12	ml. Vitalon powder
100	mg. 502 catalyst	125	mg. 502 catalyst

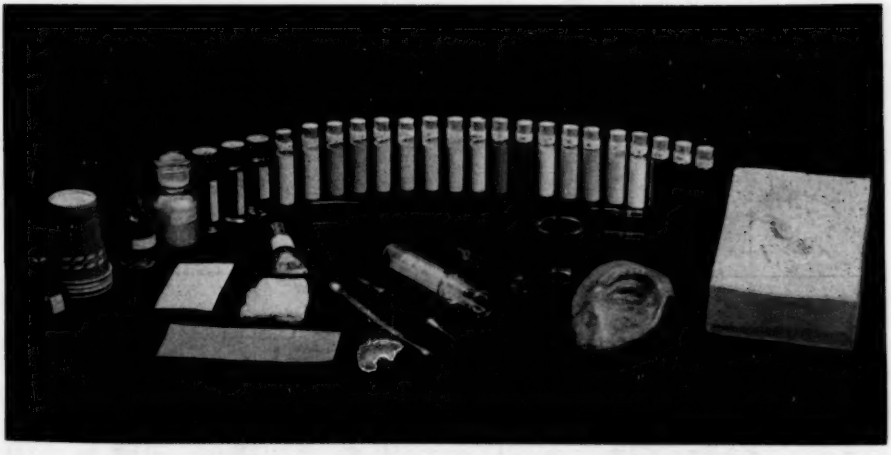

FIG. 1.—*Back row, left to right:* dental chip blower, 5-oz. paper mixing cups, 502 catalyst, Silastic RTV 502, clear separator, 200 fluid, liquid resin, color-form denture stain powders F, 1, 2, E, A, B, 4, 5. Opaque concentrated stains SDB, BR, P, Y, BL, GR, W, denture-base powder 1060M, powdered lamp black, red rayon flock, color-charted stone mold. *Left center:* white pressed fabric, nylon stocking material, wax-treated paper towel, tube of adhesive. *Top center:* pipettes, glass beaker, 10-cc. syringe, 20-cc. syringe, blade and spoon instrument, stainless-steel spatula and wax pattern with wax-treated paper towel adapted to lower half.

TABLE 2

LIGHT AND MEDIUM TISSUE COLOR FORMULAS

1 All 1	2 1 part—1 + 1 part—2	3 1 part—F + 1 part—1	4 1 part—F + 1 part—2	5 1 part—F 1 part—1 1 part—2 1 part—E	6 All F	7 1 part—1060M + 1 part—F	8 1 part—1060M + 1 part—1	9 All 1060M
10 1 part—1060M + 1 part—2	11 3 parts—2 + 1 part—F	12 1 part—1 + 1 part—E	13 1 part—F + 1 part—E	14 All 2	15 1 part—2 + 1 part—E	16 1 part—2 + 1 part—A	17 1 part—1 1 part—2 1 part—E 1 part—A	18 1 part—1 + 1 part—A
19 1 part—F + 1 part—A	20 1 part—F + 1 part—B	21 1 part—2 + 1 part—B	22 1 part—1060M + 1 part—E	23 1 part—E + 1 part—A	24 1 part—1 + 1 part—B	25 All E		

TABLE 3

RED AND PURPLE TISSUE COLOR FORMULAS

26 1 part—E + 1 part—B	27 1 part—2 1 part—E 1 part—A 1 part—B	28 All A	29 All B	30 1 part—A + 1 part—B	31 2 parts—2 1 part—F 1 part—4	32 1 part—F + 1 part—4	33 1 part—1 + 1 part—4	34 1 part—2 + 1 part—4
35 1 part—E + 1 part—4	36 1 part—A + 1 part—4	37 1 part—B + 1 part—4	38 All 4	39 1 part—F + 1 part—5	40 1 part—1 + 1 part—5	41 1 part—2 + 1 part—5	42 1 part—E + 1 part—5	43 1 part—A + 1 part—5
44 1 part—B + 1 part—5	45 1 part—4 + 1 part—5	46 All 5						

TABLE 4

BROWN TISSUE COLOR FORMULAS

47 1 part—A + 1 part—BR	48 1 part—E + 1 part—BR	49 1 part—2 + 1 part—BR	50 1 part—1 + 1 part—BR	51 1 part—F + 1 part—BR	52 All BR	53 1 part—SDB + 1 part—Y	54 1 part—F 1 part—2 1 part—4 1 part—BR	55 1 part—F 1 part—2 1 part—4 1 part—SDB
56 2 parts—4 1 part—F 1 part—SDB	57 1 part—A + 1 part—SDB	58 1 part—E + 1 part—SDB	59 1 part—2 + 1 part—SDB	60 1 part—1 + 1 part—SDB	61 1 part—F + 1 part—SDB	62 All SDB	63 1 part—VPRM 1 part—SDB 1 part—BR 1 part—P	64 1 part—VPRM + 1 part—Y
65 1 part—VPRM + 1 part—BR	66 1 part—VPRM + 1 part—SDB	67 All VPRM						

TABLE 5

PINK, YELLOW, BLUE, AND GRAY TISSUE COLOR FORMULAS

68 1 part—1 + 1 part—P	69 1 part—2 + 1 part—P	70 1 part—F + 1 part—P	71 1 part—E + 1 part—P	72 1 part—1060M + 1 part—P	73 1 part—A + 1 part—P	74 All P	75 1 part—1 + 1 part—Y	76 1 part—F + 1 part—Y
77 1 part—2 + 1 part—Y	78 1 part—E + 1 part—Y	79 1 part—A + 1 part—Y	80 1 part—B + 1 part—Y	81 1 part—P + 1 part—Y	82 1 part—4 + 1 part—Y	83 1 part—5 + 1 part—Y	84 All Y	85 1 part—BR + 1 part—Y
86 1 part—BL + 1 part—Y	87 1 part—GR + 1 part—Y	88 1 part—F + 1 part—BL	89 1 part—1 + 1 part—BL	90 1 part—2 + 1 part—BL	91 1 part—E + 1 part—BL	92 1 part—A + 1 part—BL	93 All BL	94 1 part—BL + 1 part—GR
95 1 part—F + 1 part—GR	96 1 part—1 + 1 part—GR	97 1 part—2 + 1 part—GR	98 1 part—E + 1 part—GR	99 1 part—A + 1 part—GR	100 All GR	101 Black 3 parts—VPRM + 1 part Lamp Black	102 All White	

walls. The final small amount of material adhering to the base of the plunger can be scraped off with a small, sharp instrument and allowed to fall into the center of the mass already in the bottom of the cup. Material should not be allowed to run down the walls of the cup, as its thickness will preclude all of it from joining the mass. Residual material on the cup walls may not eventually be incorporated into the mix, thereby running the risk of not reproducing the desired color exactly.

With a pipette, the desired amount of 200 silicone fluid is extracted from its container and allowed to flow into the cup containing the base material.

With a second pipette, the desired amount of liquid resin is extracted from its container and allowed to flow into the cup containing the other two ingredients.

This entire mass is then spatulated with a stainless-steel instrument until it becomes a homogenous mixture. All the material remaining on the spatula is scraped with a sharp instrument into the center of the mass in the cup.

Having previously plugged the bottom hole of a syringe with a small piece of round wood, the desired amount of methyl methacrylate powder is accurately measured, one color at a time, and emptied into a glass beaker. When all colors required by formula are in the beaker, these powders are thoroughly mixed with a stainless-steel instrument. These mixed color powders are then added to the other three materials in the cup, and this entire mass is again spatulated to a creamy, homogeneous mixture.

The 1–5 or more drops of 502 catalyst are now added to the batch with extreme care, so that the catalyst clinging to the outer walls of the eye dropper does not run down to the tip and increase the drop size. This can be avoided by wiping the outer walls of the dropper against the mouth of the catalyst container as the dropper is withdrawn.

When the desired amount of catalyst has been added to the batch in the cup, this is once again spatulated thoroughly, so that the polymerization will be completely uniform. From this, the catalyzing point, the worker has about 12 minutes to carry the desired amount of the material to the mold. This can be accomplished by picking up a quantity of the mix from the cup with a small stainless-steel instrument and spreading it in the desired charted area in the mold. Care must be exercised not to entrap air bubbles. The material can be built up to any desired depth to insure maintenance of that specific color in its particular area. The depth of material should be great enough that the base color used eventually to load the mold will not burn through and wash out any of the foreground coloring. To avoid sharp color demarcation from area to area, the edge of an area can be spread rather thin so as to blend into an adjacent color.

From this point the procedure can be repeated, adding the remaining selected colors to the mold until the mold is finally filled with the base color and closed for processing.

An excellent tissue surface can be achieved by cutting various-sized pieces of paper towel* and dipping them into a container of molten base plate wax, allowing the excess wax to flow from the towel surface. This paper can then be lightly flamed and adapted to the outer surface of the wax pattern, care being taken not to incorporate any unwanted creases. This tissue-like surface of the wax pattern is packed up by the investing stone and reproduced on the surface of the prosthesis when the silicone–methyl

* Nibroc brand, #5020, Brown Company, Berlin, N.H.

methacrylate material is applied to the surface of the negative mold from which the positive wax pattern has been flushed.

After the stone mold has been color-charted, it can be painted with a clear tin-foil substitute.* Should some separating medium adhere to the surface of the completed prosthesis upon removal from the mold, it can be washed off with water. At times, soaking in water aids in its removal.

Capillary veins can be effectively simulated by first applying a very thin coating of the colored material to the surface of the mold and then spraying the required amount of red rayon flock #100, length 0.045–5, onto this layer from a flock-loaded dental chip blower.

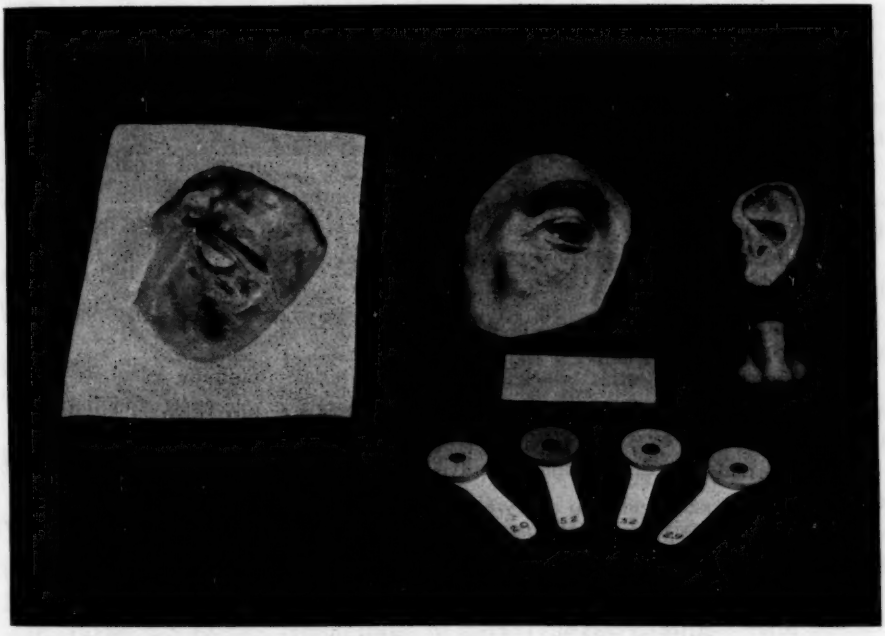

FIG. 2.—*Left to right:* stone mold with all specific-area colors in place; facial, ear, and nose prosthesis; thin, flat piece of material; and portion of color guide.

Upon expressing the base material from the syringe into the cup, the syringe should be promptly separated and cleaned in chloroform. If this is not done within a short time, it is almost impossible to remove the plunger.

The thin edges of a prosthesis can, if desired, be reinforced with a white nylon stocking material at the time the base color is being poured into the mold. The excess nylon material lying on the land of the mold beyond the edge of the mold cavity can be trimmed away from the completed prosthesis with small, sharp scissors. The nylon stocking material, being white, picks up the color of the base material very well. It is only through extremely close inspection of the outer surface of the finished piece that occasionally a fine gridwork appears. Should more ridged reinforcing be desired, the

* Modern foil, Modern Materials Mfg. Co., St. Louis, Mo.

procedure outlined above can be followed by replacing the nylon material with a white pressed fabric.*

When making the tissue color guide (Fig. 2), it is suggested that the smallest quantity of batch mix be used. The required amounts of material should be mixed in one cup and then poured into a fresh cup for room-temperature polymerization. The wax lining of the cup acts as an excellent separating agent, and the color disk can be easily removed and trimmed to any desired size or shape. The reason for changing cups is that if the mix were allowed to set up in the cup in which it is mixed, a marbelized effect would appear on the bottom surface of the finished piece. Also, all the material at the bottom edges of the cup cannot be incorporated into the batch after catalyzation, and the disk would have a slight unpolymerized edge.

DISCUSSION

It is hoped that the above description will serve as an adequate guide to the potential user and thereby provide him with the benefit of the many hours of research which have been invested in this development.

The material and technic outlined in this paper are by no means a panacea in the art of somato-prosthetics, but rather a fresh, new approach to the problem. Assuredly, the material will serve well for those cases in the oral environs, as shown in Figure 2.

The formula provides an opportunity to compound a material embodying the following features:

1. Chemically, it contains two coexisting networks when polymerized—one of the silicone rubber and one of the methyl methacrylate.

2. Its physical properties appear to offer a material containing adequate tensile strength, elongation per cent, tear strength, temperature tolerance, and durometer measurement, to serve well for its designed purpose.

3. It will maintain dimensional stability upon reaching its completed polymerized state.

a) After polymerizing for 16 hours at room temperature, the percentage of linear shrinkage was as follows:

Upon opening mold	0
After 3 days at 77° F.	2
After 6 days at 77° F.	2.1
After 14 days at 77° F.	2.1

There was a slight liquid resin odor at 3 days, but the test piece was odorless at 6 days.

b) After polymerizing for 16 hours in dry heat at 130° F., the percentage of linear shrinkage was as follows:

Upon opening mold	0
After 3 days at 77° F.	2
After 6 days at 77° F.	2.1
After 14 days at 77° F.	2.5

The test piece was odorless at 3 days.

* Interlon.

4. It has satisfactory color stability. Laboratory pieces have been observed for a period of 1 year without noticeable color change.

5. The material will remain flexible for an indefinite period of time.

6. It contains a texture which closely simulates that of human tissue.

7. The material reveals a light translucency that compares favorably with that of human tissue.

8. A processed specimen, even though quite resistant to dirt acquired through normal handling, can be easily cleaned with either soap and water, 95 per cent ethyl alcohol, carbon tetrachloride, or acetone.

9. The material can be cemented to human tissue most satisfactorily with an adhesive mixture containing 75 per cent Medico Adhesive* and 25 per cent pressure-sensitive silicone adhesive.† This adhesive mixture must be applied to the tissue-contacting surface of the material in a thin layer, allowed to set in open air for 3–4 minutes, and then the prosthesis pressed into place.

10. An original prosthesis can be relined or repaired with a new mix of material of the same color, should damage or tissue change occur. These repairs, relines, or alterations can be made with the room-temperature processing procedure.

11. The material may be polymerized by either of the two following methods: (*a*) in a closed artificial stone mold‡ at room temperature, overnight or 16 hours; (*b*) in a closed artificial stone mold, same material as above, in dry heat at 130° for overnight or 16 hours.

Note: Method *b* is used in an attempt to drive off any excess liquid resin that may be present in the material mass. The choice of the polymerizing methods seems to make no apparent difference in the physical characteristics of the completed product.

12. The color can be exactly reproduced or matched from mix to mix, provided that one maintains precision in all measurements.

13. The color is not affected by the choice of processing methods.

14. The material as formulated above is believed to be a non-toxic compound.

15. The material can be molded with standard, currently used dental laboratory technics and equipment to any form required in the prosthetic reproduction of the human body.

16. Tables 2–5 contain combinations of methyl methacrylate color-form denture stain powders—F, 1, 2, E, A, B, 4, 5. Opaque concentrated stains—(SDB) special dark brown, (BR) brown, (P) pink, (Y) yellow, (BL) blue, (GR) gray, (W) white. (VPRM) vulcanite plastic repair material. (1060M) Vitalon denture base powder 1060 tissue tone medium. Powdered lamp black.

Tylman[8] in 1944 outlined the qualities desired in materials used in maxillo-facial prostheses. It is felt that the research described herein reasonably approaches the fulfilment of these requirements. By making these findings known to all interested practitioners, broad clinical use of both material and technic will lead to further refinements in the ever increasing demand to reach greater perfection in the many aids used to serve the patient.

* Slomons Laboratories, Inc., Long Island City 1, N.Y.

† #269, Dow Corning Corporation, Midland, Mich.

‡ Kerr Rapid Stone White, Kerr Dental Mfg. Co., Detroit, Mich.

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Distribution of Fluoride in Teeth from Areas with Different Levels of Fluoride in the Water Supply *

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Previous studies have shown that fluoride occurs in greater concentrations in surface than in subsurface enamel[1-4] and that there is an increase with age in the deposition of fluoride in the surface, but not in the inner portion of the enamel.[3, 4] It has also been found that an increase in the level of water fluoride brings about an increase in the deposition of fluoride that is more marked in the surface than in the subsurface enamel.[2-4] With regard to dentin, a greater concentration of fluoride occurs in root than in crown portions; and concentrations decrease in crown dentin from the pulp toward the dentino-enamel junction.[5] The above studies, together with observations using radioactive fluoride,[6-9] suggest that fluoride accumulates in the surface structures of teeth and that there may be a marked difference in the fluoride distribution not only in enamel and crown dentin but also, by inference, in the root portion. The difference in character of the various tooth surfaces is likely to affect the fluoride uptake. Therefore, the present study was undertaken to investigate further the deposition of fluoride in surface and subsurface material. Concentrations of fluoride were determined in layer samples of enamel, crown dentin, and root dentin of teeth of varying ages from different communities with known, but different concentrations of fluoride in the water supply.

MATERIALS AND METHODS

Erupted permanent teeth from persons who had resided continuously in the communities in which they were born were selected for study. The teeth were divided into the following groups according to the age of the patient: under twenty, twenty to twenty-nine, thirty to forty-nine, and over fifty years. Table 1 lists the communities, the concentration of fluoride in the drinking water of each community, the age of the teeth, the total number of teeth, the number of anteriors and posteriors, and the number of sound and carious teeth in each group.

After the teeth were cleaned, a shallow groove was cut along the cemento-enamel junction to prevent inclusion of cementum in the grinding of the enamel samples. The first three thin layers of enamel were ground according to the method previously described.[3] The teeth were then split longitudinally in two or more portions, and the

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TABLE 1

LOCATIONS, CONCENTRATIONS OF FLUORIDE IN WATER SUPPLY, AGE, AND TOTAL NUMBER, AS WELL AS NUMBER OF ANTERIOR AND POSTERIOR TEETH, AND SOUND AND CARIOUS TEETH OF GROUPS STUDIED

COMMUNITIES	F CONCEN. (PPM) IN WATER	AGE GROUPS (YEARS)	TOTAL NO. OF TEETH	NUMBER OF			
				Anterior	Posterior	Sound	Carious
Doland, S.D.	2.9	<20	27	7	20	.....	27
Big Lake, Tex.	3.0	<20	8	2	6	7	1
McKinney and Center, Tex.	1.0	20–29	26	3	23	7	19
Doland, S.D.	2.9	20–29	14	3	11	2	12
Big Lake, Tex.	3.0	20–29	22	6	16	17	5
Center, Tex.	1.0	30–49	10	5	5	8	2
Slaton, Tex.	5.2	30–49	31	22	9	23	8
Chicago, Ill.	0.1	>50	41	27	14	11	30
Doland, S.D.	2.9	>50	85	48	37	53	32
Big Lake, Tex.	3.0	>50	10	1	9	7	3

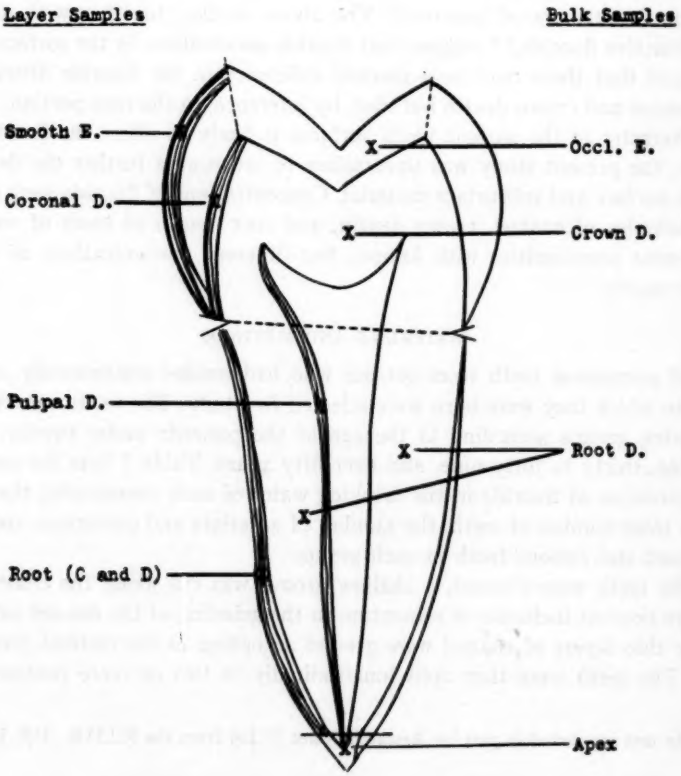

FIG. 1.—Diagram showing how the teeth were sampled

visible dentino-enamel junction was used as a further guide for grinding. First, a bulk sample of occlusal enamel was collected, and then a fourth relatively thick layer was ground a uniform distance from the dentino-enamel junction. Two additional layers (the fifth and the sixth) were ground from the remaining enamel, the last layer including materials from the dentino-enamel junction. The samples were examined for dentin contamination by observing the position of the Becke line of particles submerged in aniline (refractive index 1.585). Samples having less than 96 per cent enamel were purified by the flotation method.[10, 11]

Dentin was sampled as shown in Figure 1. Four successive layers of coronal dentin were ground from the dentino-enamel junction inward, the last layer having a greater

TABLE 2

DRY WEIGHT (MILLIGRAMS) OF SUCCESSIVE LAYER SAMPLES OF TEETH OF PERSONS UNDER 20, 20–29, 30–49, AND OVER 50 YEARS OLD FROM COMMUNITIES WITH 0.1, 1.0, 2.9, 3.0, AND 5.2 ppm FLUORIDE IN WATER SUPPLY

	<20 Years		20–29 Years			30–49 Years		>50 Years		
Layers*	2.9 ppm F Doland, S.D.	3.0 ppm F BigLake, Tex.	1.0 ppm F McKinney and Center, Tex.	2.9 ppm F Doland, S.D.	3.0 ppm F BigLake, Tex.	1.0 ppm F Center, Tex.	5.2 ppm F Slaton, Tex.	0.1 ppm F Chicago, Ill.	2.9 ppm F Doland, S.D.	3.0 ppm F BigLake Tex.
OE	2,615	829	1,980	1,442	2,233	658	1,900	.....	3,015	1,069
E-1	166	90	186	100	386	73	222	236	783	71
E-2	130	65	158	91	321	76	154	206	347	83
E-3	118	72	200	132	296	51	151	218	376	67
E-4	2,358	1,166	2,690	1,586	2,491	938	3,066	.....	4,646	1,225
E-5	131	58	269	66	119	73	248	687	557	90
E-6	106	65	159	110	187	41	195	267	371	57
DE-J	1,705	660	1,937	1,086	1,300	724	3,040	.....	6,427	740
D-1	95	65	106	76	160	70	185	91	292	63
D-2	96	46	128	54	108	42	168	64	214	64
D-3	102	51	104	44	70	52	166	19	171	43
D-4	115	124	114	157	190	149	133	31	687	121
D-5	.....	.....	271	.....	.....	.....	943	150	.....	.....
CD	3,422	1,066	928	2,235	4,410	2,125	1,264	869	.....	1,390
PD-4	.....	83	304	59	.....	43	296	.....	.....	45
PD-3	61	16	40	23	91	15	64	.....	120	12
PD-2	56	17	44	17	53	15	85	79	120	16
PD-1	75	20	106	26	139	16	115	107	100	23
R-1	169	73	143	117	287	99	206	186	332	86
R-2	111	49	137	91	179	91	164	114	251	85
R-3	85	77	134	126	157	92	201	147	210	89
R-4	.....	192	472	389	.....	301	.....	152	256	311
R-5	.....	.....	.....	.....	.....	.....	.....	137	245	.....
RD	10,850	4,000	.....	8,200	10,153	3,468	.....	10,400	.....	4,100
Apex	245	28	245	105	229	26	259	.....	2,151	.....

* Letters in this column have the following meanings: OE—occlusal enamel, E—enamel, DE-J—dentino-enamel junction, D—dentin, CD—crown dentin, PD—pulpal dentin, R—root, RD—root dentin.

thickness than the others. Three layers of dentin from the pulp side (pulpal dentin) were obtained by means of a round carbide bur. Layer samples were also ground from the surface of the root. No attempt was made to separate cementum and dentin in the root samples. The first and second root layers consisted mostly of cementum, while the remaining layers consisted predominantly of dentin. Bulk samples of the crown and root dentin and of the root tips were also included for analysis.

The weights of the samples are shown in Table 2. In order to facilitate comparison of the data, an attempt was made to grind corresponding layers in each group to the same thickness. The thickness of the enamel layers was assessed by employing the method previously described.[3] The thickness indices and the surface scores of enamel are presented in Table 3.

TABLE 3

THICKNESS INDICES AND SURFACE SCORES OF SUCCESSIVE LAYER SAMPLES OF ENAMEL OF PERSONS UNDER 20, 20–29, 30–49, AND OVER 50 YEARS OLD FROM COMMUNITIES WITH 0.1, 1.0, 2.9, 3.0, AND 5.2 ppm FLUORIDE IN WATER SUPPLY

Layers	<20 Years		20–29 Years			30–49 Years		>50 Years		
	2.9 ppm F Doland, S.D.	3.0 ppm F BigLake, Tex.	1.0 ppm F McKinney and Center, Tex.	2.9 ppm F Doland, S.D.	3.0 ppm F BigLake, Tex.	1.0 ppm F Center, Tex.	5.2 ppm F Slaton, Tex.	0.1 ppm F Chicago, Ill.	2.9 ppm F Doland, S.D.	3.0 ppm F BigLake, Tex.
E-1	4.0	4.7	3.7	3.8	7.8	3.7	4.4	3.6	7.8	3.6
E-2	3.1	3.4	3.1	3.5	6.6	3.7	3.1	3.2	3.5	4.2
E-3	2.8	3.8	4.0	5.1	6.0	2.5	3.0	3.4	3.8	3.4
E-4	56.1	61.3	53.8	61.1	50.8	46.9	61.2	.....	46.5	61.2
E-5	3.1	3.1	5.4	2.5	2.4	3.7	5.0	10.6	5.6	4.5
E-6	2.5	3.4	3.2	4.2	3.8	2.1	3.9	4.1	3.7	7.9
Surface score	42	19	50	26	49	20	50	65	100	20

For the determination of fluoride, the samples were ashed in platinum dishes at 575° C. and weighed. The fluoride was separated by the distillation method of Willard and Winter.[12] The fluoride in the distillate was determined by the titration method of Smith and Gardner.[13]

RESULTS

The results are summarized in Table 4. The first line gives the fluoride concentrations of bulk samples of occlusal enamel. There is an increase in concentration with an increase in fluoride in the drinking water, but no consistent change with age in these bulk samples. In lines 2 through 8 the concentration of fluoride in succeeding layers of smooth surface enamel are listed. Concentrations are high in the outermost layer of all tooth groups, and there is a marked decrease from the surface inward. In the middle and inner third of the enamel, concentrations tend to level off, with small deviations occurring adjacent to the dentino-enamel junction.

Comparison of similar age groups from different geographical areas shows an increase in fluoride deposition with increasing levels of fluoride in the water supply.

This increase, apparent in all layers of enamel, is illustrated in Figure 2, where concentrations of successive layers of the twenty to twenty-nine age groups are plotted against the depth from the surface. Approximately two times greater concentrations of fluoride are deposited in the enamel of teeth from communities with 3.0 or 2.9 ppm of fluoride in the water than in enamel from 1-ppm areas. The similar pattern of distribution of fluoride in the different enamel groups can also be seen in this graph.

The distribution of fluoride in crown dentin from the dentino-enamel junction to the pulp is given in lines 9 through 15 in Table 4. The greatest concentrations were always found in the dentin adjacent to the pulp. The level of dentinal fluoride usually

TABLE 4

FLUORIDE (ppm ASH WEIGHT) OF SUCCESSIVE LAYER SAMPLES OF TEETH OF PERSONS UNDER 20, 20–29, 30–49, AND OVER 50 YEARS OLD FROM COMMUNITIES WITH 0.1, 1.0, 2.9, 3.0, AND 5.2 ppm FLUORIDE IN WATER SUPPLY

LAYERS*	<20 YEARS		20–29 YEARS			30–49 YEARS		>50 YEARS		
	2.9 ppm F Doland, S.D.	3.0 ppm F BigLake, Tex.	1.0 ppm F McKinney and Center, Tex.	2.9 ppm F Doland, S.D.	3.0 ppm F BigLake, Tex.	1.0 ppm F Center, Tex.	5.2 ppm F Slaton, Tex.	0.1 ppm F Chicago, Ill.	2.9 ppm F Doland, S.D.	3.0 ppm F BigLake, Tex.
OE	127	273	163	215	305	117	516	.....	171	196
E-1	1,773	1,930	1,087	1,957	2,100	1,132	3,707	1,080	1,697	2,290
E-2	803	1,010	591	1,193	918	360	2,340	540	1,084	993
E-3	813	992	319	853	448	230	1,772	305	683	600
E-4	165	262	87	217	222	149	538	.....	109	94
E-5	183	353	147	452	299	91	518	.....	100	289
E-6	245	152	98	329	240	.....	538	60	93	227
E	99	177	64	147	287	57	453	.....	132	143
DE-J										
D	673	870	255	1,540	933	139	1,680	.....	.....	637
D-1	581	720	301	688	991	442	2,000	567	454	699
D-2	948	980	235	822	932	216	1,700	247	690	732
D-3	563	1,268	283	1,105	799	221	1,650	.....	540	596
D-4	636	991	220	523	722	237	1,980	.....	422	644
D-5	.....	.....	233	.....	.....	.....	1,622	231	.....	.....
CD	683	991	.....	843	1,280	358	2,690	241	1,742	1,285
PD-4	.....	.....	1,230	.....	.....	1,371	6,270	.....	.....	5,280
PD-3	3,070	2,010	1,540	2,650	4,390	1,369	8,450	.....	7,980	6,350
PD-2	3,190	3,580	1,530	3,090	5,233	1,910	8,810	1,654	8,680	6,340
PD-1	2,840	3,400	1,662	3,660	5,385	2,340	8,150	1,901	8,637	6,600
R-1	4,010	3,690	2,330	4,370	4,912	2,050	8,080	2,387	9,037	6,850
R-2	3,440	3,070	1,972	2,850	4,384	1,725	9,360	2,430	8,836	5,290
R-3	3,030	2,170	1,607	2,700	3,138	1,192	7,410	2,305	8,964	3,700
R-4	.....	1,578	1,058	1,783	.....	718	.....	1,945	8,115	1,790
R-5	.....	.....	.....	.....	.....	.....	.....	1,886	7,231	.....
RD	1,193	1,143	.....	1,652	1,587	393	3,030	578	2,871	1,400
Apex	1,937	1,007	947	2,010	2,837	1,313	4,950	.....	4,100	4,200

* See footnote to Table 2 for meanings of letters in this column.

decreased from the pulp outward, at first slightly, then more abruptly until a plateau was reached in the middle third, with concentrations ranging from one-fifth to one-half those in the pulpal layers. Except for small variations, no further change was found in the fluoride level in the outer portion of the crown dentin.

There was increased deposition of fluoride in the crown dentin with an increase in the fluoride level of the water supply, as shown in Figure 3, where the distribution of fluoride is plotted for the age groups thirty to forty-nine from 1.0- to 5.2-ppm fluoride areas. Aside from the marked differences in concentration, the pattern of distribution is remarkably similar in the two groups. The marked variation in fluoride deposition in dentin from different communities is illustrated by the wide range found in pulpal dentin samples, the maximal concentration being the 8,810 ppm in the thirty to forty-nine age group from Slaton, Texas, and the minimal concentration the 1,662 ppm in the twenty to twenty-nine group from McKinney, Texas (5.2 and 1.0 ppm of fluoride, respectively).

It will be noted in Table 4 that there is a marked increase with age in the concentration of fluoride in the pulpal dentin. For example, the figures for the under twenty, twenty to twenty-nine, and over-fifty groups from Big Lake, Texas, were 3,400, 5,385, and 6,600 ppm, respectively. The age changes were always conspicuous in the surface layers of dentin but were not noticeable in the dentin adjacent to the enamel.

Graphs of the distribution of fluoride in the crowns of teeth from the under twenty group, from areas with 2.9 and 3.0 ppm of fluoride in the water are given in Figure 4. In both groups there are high concentrations of fluoride in the outermost layers of the enamel, a rapid decrease in subsurface enamel, followed by a leveling-off and a final slight decrease at the dentino-enamel junction. Coronal dentin shows substantially higher fluoride concentrations than adjacent enamel, but the distribution is reminiscent of that of enamel with the lowest levels of fluoride in bulk dentin and maximal concentrations at the pulpal surface.

The concentrations of fluoride in five successive layers of the roots and root tips from different tooth groups are given in the last six lines of Table 4. The external portions of the roots are consistently high in fluoride, and there is a decrease in concentration from the surface inward, with minimal levels occurring approximately midway between the root surface and the pulp (layer RD in Table 4). Figure 5 shows the distribution of fluoride from the root surface to the pulp in the thirty to forty-nine groups from 5.2- to 1.0-ppm fluoride areas. In both groups maximal concentrations occur at the pulpal and root surfaces and minimal concentrations in the middle portion. The marked increase in fluoride deposition in the roots with an increase in fluoride in the water supply is also apparent.

Figure 6 shows the effect of age on fluoride deposition in roots from 3.0-ppm fluoride areas. The under twenty group has less fluoride in all portions of the root than do the older age groups. The twenty to thirty-nine and the over fifty groups are similar except that the external and pulpal layers show higher fluoride levels in the older groups.

The last line in Table 4 shows the concentrations of fluoride in the tip of the roots. In all groups except one, this portion contained more fluoride than did bulk samples of crown or root dentin.

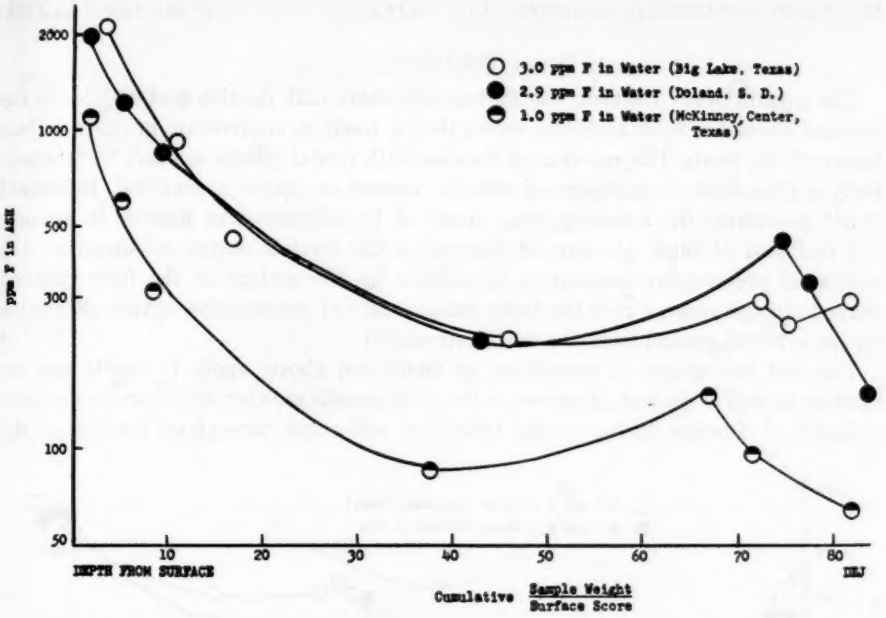

FIG. 2.—F (ppm) in successive layers of enamel of teeth of persons twenty to twenty-nine years, from areas with 1.0, 2.9, and 3.0 ppm F in the water supply.

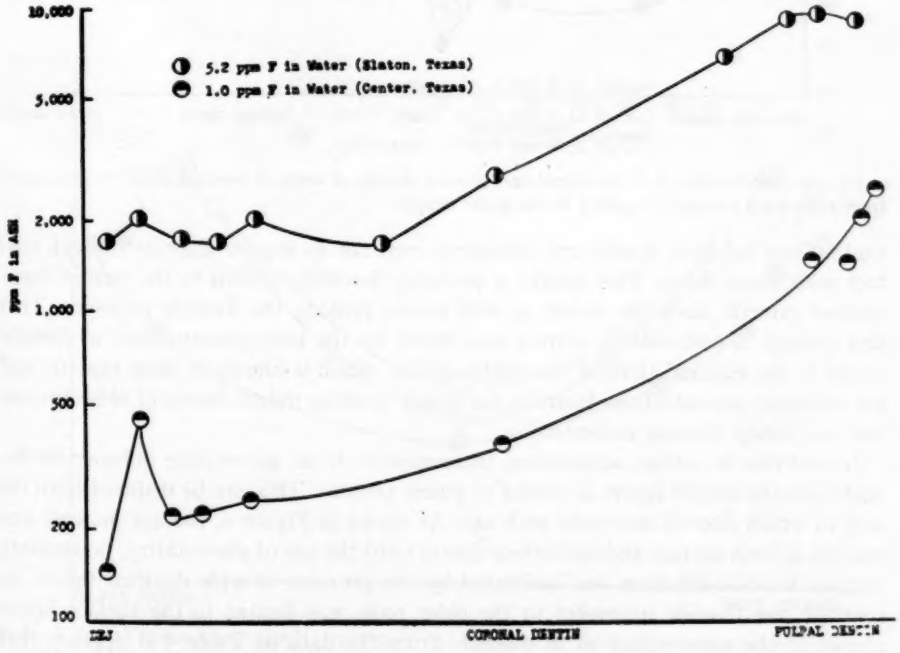

FIG. 3.—F(ppm) in successive layers of enamel of teeth of persons thirty to forty-nine years, from areas with 1.0 and 5.2 ppm F in the water supply.

DISCUSSION

The present study confirms the findings of others that fluoride accumulates in the external enamel and, in addition, shows that it tends to concentrate in other surface layers of the teeth. The reaction of fluoride with dental tissues appears to be essentially surface-limited regardless of whether enamel or dentin is involved. Brudevold *et al.*[3] postulated the following three stages of the deposition of fluoride in enamel: (1) inclusion of small amounts of fluoride in the crystals during calcification; (2) additional pre-eruptive acquisition of fluoride by the surface of the fully calcified enamel through contact with the tissue fluids; and (3) posteruptive uptake of fluoride by the external enamel from the oral environment.

The first two stages of deposition, as mentioned above, apply to dentin and cementum as well as enamel. However, in the continuously growing dentin and cementum, inclusion of fluoride during crystal formation will occur throughout the life of the

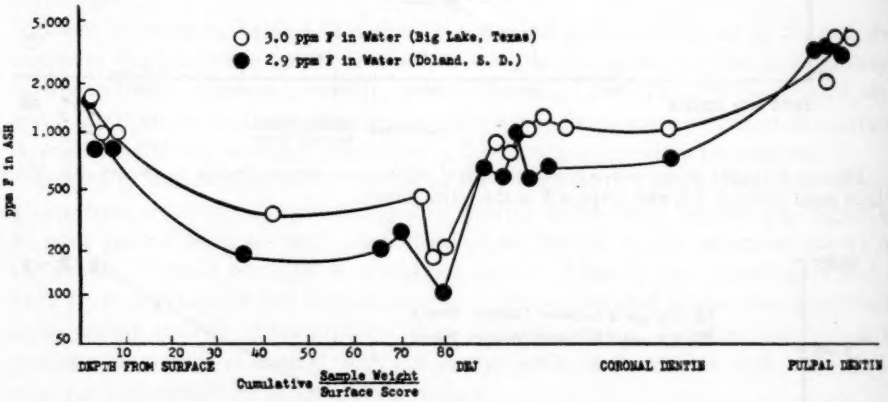

FIG. 4.—Distribution of F in enamel and coronal dentin of teeth of persons under twenty years, from areas with 2.9 and 3.0 ppm F in the water supply.

tooth. Once calcified, dentin and cementum continue to acquire fluoride through contact with tissue fluids. This uptake is probably inversely related to the rate of appositional growth, since the slower growth should prolong the fluoride exposure. That this concept is undoubtedly correct was shown by the low concentrations of fluoride found in the external third of the crown dentin, which is known to form rapidly, and the maximal concentrations found in the slowly growing pulpal dentin of older persons and the slowly forming cementum.

In addition to surface acquisition, there appears to be appreciable diffusion of fluoride into the deeper layers of dentin of young persons. This can be deduced from the way in which fluoride increased with age. As shown in Figure 6, the age increase was marked in both surface and subsurface dentin until the age of about thirty, presumably because fluoride diffusion was facilitated by the presence of wide dentinal tubuli. In contrast, the fluoride increment in the older roots was limited to the surface layers almost to the same extent as in enamel. From the data in Table 4 it appears that fluoride may also diffuse from the pulp into the adjacent crown dentin. However, no increase in fluoride with age could be found in the dentin adjacent to the enamel.

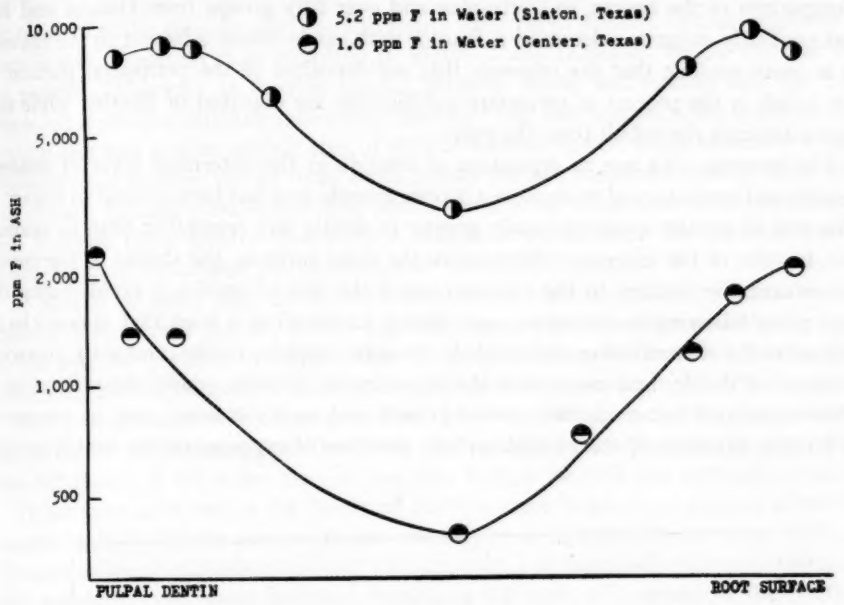

FIG. 5.—F (ppm) in successive root layers of teeth of persons thirty to forty-nine years, from areas with 1.0 and 5.2 ppm F in the water supply.

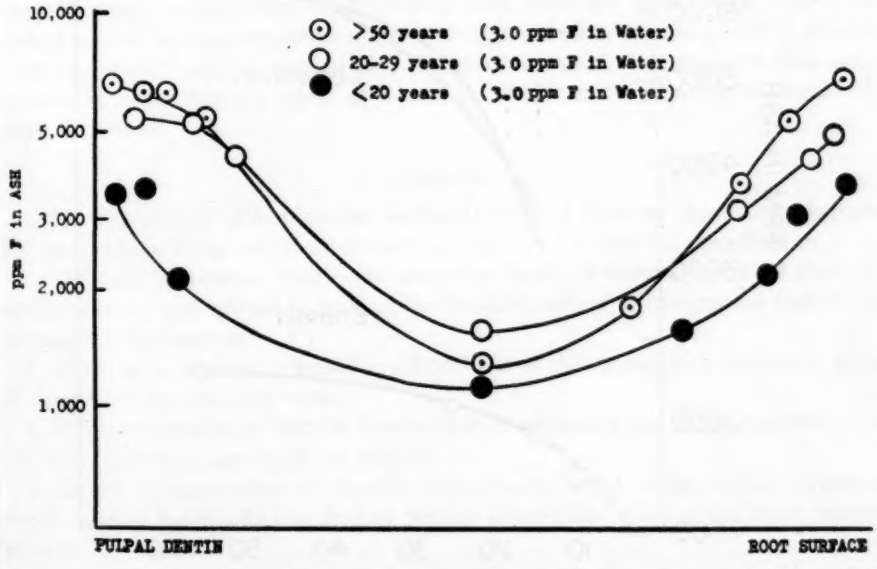

FIG. 6.—F (ppm) in successive root layers of teeth of persons under twenty, twenty to twenty-nine, and over fifty years, from areas with 1.0 and 5.2 ppm F in the water supply.

Comparison of the twenty to thirty-nine and over fifty groups from Doland and Big Lake actually suggests a decrease in fluoride with age in dentin adjacent to the enamel. It is quite possible that the minerals that are deposited in the peripheral portion of the tubuli in the process of secondary calcification are deprived of fluoride while diffusing through the tubuli from the pulp.

The increase with age in deposition of fluoride in the outermost layer of enamel, dentin, and cementum of teeth from a 3-ppm fluoride area has been plotted in Figure 7. The rate of uptake is conspicuously greater in dentin and cementum than in enamel, but, in spite of the inherent differences in the three surfaces, the shapes of the curves are remarkably similar. In the surface enamel the rate of uptake is rapid during the first years following its formation, and then it tapers off at a level that appears to be related to the concentration of fluoride in the water supply. In the constantly changing surfaces of dentin and cementum the exposure to fluoride would depend on such afore-mentioned factors as the rate of growth and ionic diffusion, and, as compared to fluoride exposure of the enamel surface, the time of exposure of the dentin surface

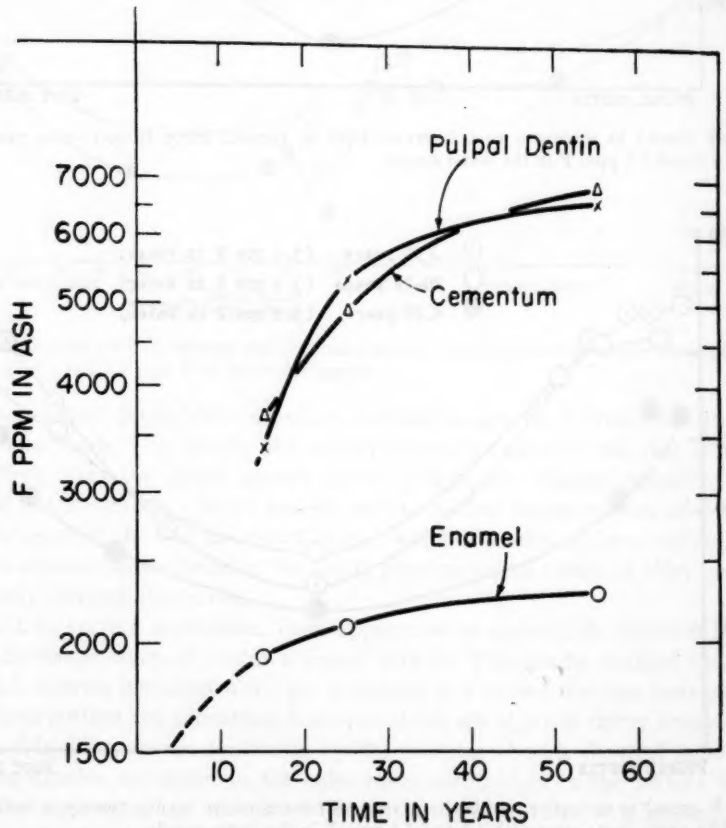

FIG. 7.—Rate of F uptake in surface layer of enamel, dentin, and cementum of teeth from areas with 3 ppm F in the water supply.

material must be considerably shorter. Hence the rate of fluoride uptake is greater in dentin and cementum than indicated in Figure 7 and obviously by far exceeds that of enamel. Difference in fluoride uptake between dentin and enamel also has been observed in studies utilizing radioactive fluoride[14] and has been ascribed to the smaller crystal size and greater surface area of dentin. Gas-adsorption measurements[15-17] have shown dentin to have two to three times greater surface area than enamel, and, since the present findings show that pulpal dentin has fluoride concentrations approximately two to three times greater than surface enamel, it would seem that most of the deposited fluoride is located at the surface of the apatite crystals.

It should be noted that dentin does not always contain greater concentrations of fluoride than enamel. In fact, greater concentrations of fluoride were consistently found in the external portions of enamel than in the bulk of crown and root dentin, demonstrating the importance of accessibility and time of exposure in fluoride deposition.

The good correlation in the present study between fluoride in the water and fluoride deposition in the teeth suggests that the major variable in fluoride ingestion stems from the differences in the water fluoride and that dietary fluoride was relatively constant.

It has been indicated in the literature that the more frequent occurrence of mottled enamel in high-fluoride areas with hot climates than in high-fluoride areas with cold climates is due to differences in water consumption. For example, a report by Galagan[18] has suggested that water supplies containing 0.6 ppm of fluoride in the southern states may be considered as effective as 1.2 ppm of fluoride in the northern states and that the fluoride level of the drinking water in different geographical areas should be adjusted to climatic conditions. In agreement with this thesis, the present findings showed greater concentrations of fluoride in teeth from Big Lake, Texas, than from Doland, South Dakota, two communities with approximately the same level of fluoride in the water (3.0 and 2.9, respectively). However, it would be desirable to have additional data on fluoride deposition as related to fluoride in the drinking water and climatic conditions.

SUMMARY

1. The distribution of fluoride was studied in teeth of different ages from geographical areas whose water supplies contain 0.1, 1.0, 2.9, 3.0, and 5.2 ppm fluoride.

2. The concentration of fluoride in successive layers of enamel decreased from the surface inward and tended to level off in the mid-portion of the enamel toward the dentino-enamel junction.

3. The level of fluoride increased in all portions of the enamel with increasing levels of fluoride in the drinking water.

4. The concentration of fluoride increased with advancing age in the outermost, but not in the principal portion of the enamel.

5. Higher concentrations of fluoride were always found in the surface layers of dentin (pulpal dentin) and in the root surface (cementum) than in the inner dentinal portions.

6. A greater increase in fluoride was observed in dentin than in enamel with increasing concentrations of fluoride in the drinking water.

7. There was a marked increase with age in the concentrations of fluoride in the pulpal portion of dentin and in the root surface.

8. The concentration of fluoride in dentin was greater than in enamel, with the exception that more fluoride was found in the outermost portion of the enamel than in the bulk portion of dentin.

The co-operation of dentists who collected the teeth used in this study is greatly appreciated.

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Modified Apparatus for the Formation of Synthetic Calculus

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The formation of a synthetic calculus-like material by using a bovine submaxillary gland extract (Draus and Leung, *Oral Surg., Oral Path. Oral Med.*, **13**:224, 1960) in place of saliva in the dipping apparatus of Leung (*J. Peridont.*, **28**:217, 1957) has been demonstrated. We wish to present here a modification of this apparatus in which the substrate solution drips over the forming medium. We feel that this condition more accurately resembles the condition in the oral cavity.

The apparatus (Fig. 1) consists essentially of a plexiglass box in which teeth or plastic pellets are mounted, a reservoir flask containing the synthetic saliva solution, and a pumping system for recycling the solution. For operation, the solution is placed in the flask (*A*) and allowed to run through the manifold (*B*) and drip over the forming medium (*C*) at a constant rate, which is controlled by clamp (*D*). The solution is then recycled by being pulled into a two-way suction bulb (*E*) (Fisher Cat. No. 14-087) and forced back into reservoir flask *A* by means of a force exerted on the bulb by a motor-driven arm.

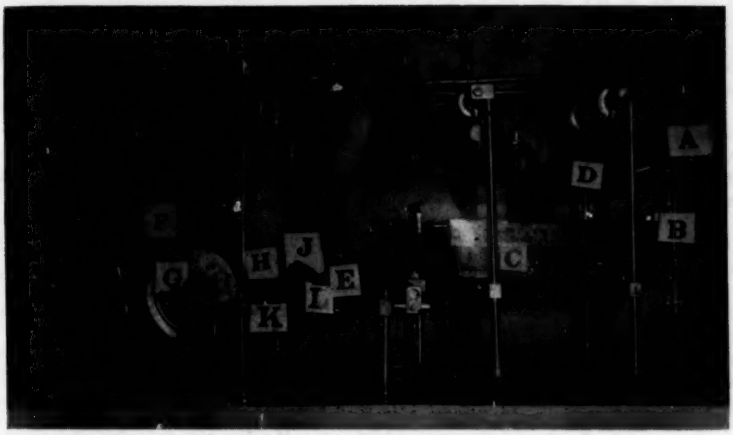

FIG. 1.—A modified apparatus for formation of synthetic calculus

The drive system consists of a ¼-horsepower motor (*F*) (Bonidine Electric Co., Chicago, Ill.) with a reduction gear set to give 43 r.p.m. This is further reduced by a series of wheels (*G*) to give 4 r.p.m. The driving arm (*H*) is connected to a horizontal bar (*J*) mounted in guide rods (*K*) on both ends, to keep the bar moving in a straight line. Two pipes (*L*) mounted on the guide rods allow the horizontal bar (*J*) free movement.

By this recycling process it is possible to minimize the amount of solution used per day.

In a typical run for the deposition of synthetic calculus, a solution containing 18 mg. per cent phosphorous, 7 mg. per cent calcium, and 0.1 per cent bovine gland extract was allowed to drip over plastic pellets for 3 weeks. The solutions were changed daily. A deposit began to form after 4–5 days.

The deposit obtained after 3 weeks resembled the material obtained from the dipping apparatus and was held strongly by the forming medium. An analysis of the deposit showed 50 per cent organic material, 31.5 per cent calcium, and 15.9 per cent phosphorous. Although the organic content is high, the inorganic component is well within the ranges reported; synthetic calculus by dipping technic: organic, 32 per cent; calcium, 30.5 per cent; and phosphorus, 17.8 per cent; and for clinical calculus: organic, 12.9–28.4 per cent; calcium, 30.7–39 per cent; and phosphorous, 16.8–20.5 per cent.

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Metabolic Availability of Fluoride from Oyster Shells

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Previous studies in our laboratories have shown that fluoride (as sodium fluoride) is less available for storage in the skeleton when furnished in milk than when added to aqueous solutions (*J. Nutrition,* **55**:347, 1955). Similar conclusions have recently been reached by Ericsson (*Acta Odont. Scand.,* **16**:51, 1958). It has been suggested that the unavailability of fluoride in milk is due to the formation of CaF_2. It seemed possible that other forms of a calcium fluoride compound, such as oyster shells, might provide fluoride in a more metabolically active form, since it not only is a practically pure phosphorus-free calcium carbonate* but contains many trace elements which may enhance fluoride availability. A total of 24 weanling Sprague-Dawley strain rats which were bred and raised in our laboratories were divided into 4 groups and received one of the 4 diets, the composition of which has previously been reported (*J. Nutrition,* **54**:481, 1954), in order to test this hypothesis. The animals in group 1 were controls and received no added fluoride. Those in group 2 received fluoride in the form of oyster shells; group 3, sodium fluoride; and group 4, calcium fluoride. These diets were designed so that the 3 experimental groups (2, 3, and 4) received the same amount of fluoride, and, upon analysis, the diets were found to contain 44.6, 45.0, and 45.7 μg F/gm, respectively. Diet 1 had a fluoride content of 1.64 μg/gm. To insure that all the groups received equal quantities of fluoride, they were all given 5 gm. of food at 8:00 A.M. each morning. As soon as each animal had eaten all of the diet, the empty food cup was removed from the cage, and no other dietary supplement was provided until the following day. Fluoride-free water was available ad libitum.

Metabolism studies were conducted daily on each animal, and fluoride, calcium, and phosphorus analyses were performed on feces and urine by methods previously described (*J. D. Res.,* **34**:900, 1954).

All animals were housed individually in raised screen cages in an air-conditioned room. The duration of the study was 30 days, after which the animals were sacrificed and the femur and whole carcass analyzed for fluoride by methods previously described (*J. Nutrition,* **54**:437, 1954).

The data show that the feces of the control group contained an average of about 15 mg. per cent Ca, 5 mg. per cent P, and 3.4 ppm F per day, while the urine contained considerably less Ca and P and only a slightly higher amount of F. The feces of the animals which received F daily from oyster shells contained 128 ppm F, and the urine contained very nearly the same amount of F (5.38 ppm) per day as the urine of the control animals. The animals which received the same amount of F as NaF contained less F in the feces (17 ppm) but significantly more in the urine (19.2), while those which received the fluoride as CaF_2 contained considerably more F in the urine than the controls (9.6 ppm) but significantly more in the feces (47 ppm).

The control carcass contained 42 μg F, while the animals receiving fluoride in the form of oyster shell contained 95 μg F. When similar amounts of fluoride were furnished as NaF, the carcass contained 628 μg F, and when the fluoride was present in the diet as CaF_2, the whole carcass contained 354 μg F.

These data clearly demonstrate the non-availability of fluoride when furnished in the form of oyster shells. When the oyster shell furnished the same concentration of fluoride as furnished by sodium fluoride, not only was less fluoride absorbed (as evidenced by high amount of fluoride in feces and low concentration in urine), but there was significantly less ($p = 0.0001$) in the carcass. Fluoride as calcium fluoride was metabolized to a greater degree than fluoride furnished as oyster shell, but not to the same extent as that furnished as sodium fluoride.

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This study was supported in part by a grant from The Marion Laboratories, Inc., Kansas City, Missouri.

* Composition of oyster shell is (in ppm) Fe, 500; Mn, 5; Cu, 5; Na, 500; K, 100; Si, 100; and fluoride, 50. Calcium, 38.8 per cent; magnesium, 0.26 per cent; and phosphorus, 0.02 per cent.

Stop-Flow Analysis of Salivary Gland Duct Transport Localization

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The stop-flow technic of Malvin, Sullivan, and Wilde (*Physiologist*, **1**:58, 1957) was employed to investigate the localization of transport mechanisms of sodium and potassium in specific areas of the acinar duct system in the submandibular gland of the dog.

The ducts were occluded for 2- and 8-minute periods on the same glands of each of five dogs. The time was kept to the nearest second, and Na and K were assayed by standard flame spectrophotometric methods. The stimulus was 0.015 mg of pilocarpine/kg of body weight. Each sample in this study represents 0.35 ml. of saliva. In each study, samples 1 and 2 represent steady-state values, while sample 6 represents an early phase of the new steady state as the gland recovers. Samples 3, 4, 5, and 6 were collected immediately following the period of occlusion.

The average values for the five dogs are presented in the table showing Na and K concentrations, Na/K ratios, and flow rates.

Sample No.	mEq/l Na	mEq/l K	Na/K	Flow Rate (ml/min)
	Duct Occluded 2 Minutes			
1.....	19.12	8.56	2.23	0.50
2.....	19.40	8.54	2.27	0.51
3.....	21.31	11.65	1.83	3.56
4.....	81.90	12.98	6.31	1.29
5.....	70.14	9.73	7.21	0.58
6.....	27.72	9.35	2.96	0.51
	Duct Occluded 8 Minutes			
1.....	23.05	7.78	2.96	0.54
2.....	24.19	7.64	3.17	0.50
3.....	30.11	11.94	2.52	3.43
4.....	102.56	8.76	11.71	1.61
5.....	91.43	4.89	18.70	0.69
6.....	51.38	6.11	8.41	0.51

In keeping with the hypothesis that the ducts will have an increased effect on a standing column of secretion, the K values of sample 3 were significantly higher than sample 2. Sample 3 showed only a very slight increase in Na over the previous samples. The large increase in Na appeared first in sample 4, at a time when the potassium concentration was beginning to be reduced. Sample 5 maintained a rather high level of sodium concentration, while K in samples 5 and 6 fell significantly, thereby giving rise to high Na/K ratios, approaching those of plasma. In keeping with the second hypothesis, that the column of saliva passed through the duct too rapidly for the duct to have its usual effect on the salivary components, we believe that the sodium concentration of the salivary components in sample 4 approached the secretion of the acini. The minimum K concentration was observed in the fifth sample of the 8-minute stop-flow periods.

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The Effect of Weaning on the Incidence of Incipient Carious Lesions

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A variety of conditions imposed upon the rat have been found to alter the incidence of dental decay. It would appear that early and profound changes in dental tissues occur in considerable depth preceding actual cavitation (Steinman, Hewes, and Woods, *J. D. Res.*, **38**:592, 1959). The weaning process as brought about by the dam is a gradual process. Unless the young are separated from the dam, nursing continues for a number of days past the usual weaning date imposed by this laboratory. The following experiment was designed to determine the effect of sudden weaning upon the incidence of incipient carious lesions in the young rats.

Sixty Osborne-Mendel rats from nine different litters were utilized in this study. Throughout their lifetime all animals were on a cariogenic diet of the following composition: casein, 24; sucrose, 65; corn oil, 5; salts U.S.P. XIV, 4; dehydrated liver, 4; vitamin mix, 2; and choline, 0.2 parts. Half the animals in each litter were weaned at 20 days of age and continued on the cariogenic diet. Weaned littermates were kept together in separate cages. The other half of the animals remained with the dams on the same cariogenic diet. All animals were sacrificed at 30 days of age. The jaws were frozen immediately and sectioned with a microtome in a deep freeze.

The sections were studied histochemically after fixing in alcohol and treating with 0.5 per cent ninhydrin for 18 hours at 37° C. The blue color produced by the ninhydrin will gradually disappear unless the sections are kept in a deep freeze. The sections were studied microscopically to determine the incidence of incipient lesions (Steinman *et al.*, *J. D. Res.*, **38**:592, 1959).

There was no significant difference in weight between the two groups of animals at 30 days of age, at which time all animals were sacrificed. The 30 animals weaned at 20 days of age had an average of 8.3 incipient lesions per animal, whereas the 30 littermates which were allowed to remain with the dams until sacrificed at 30 days of age had an average of 3.6 incipient lesions per animal. The results of this study are summarized in the table.

Days Fed Cariogenic Diet	Age Weaned	No. of Animals	No. of Lesions	Average
0–30.....	20	30	249	8.3
0–30.....	Not weaned	30	108	3.6

The chi-square analysis of these data gives a probability of the order of 1,000 to 1 that the observed difference was not due to chance.

One would suspect the observed reduction in caries reported in this study to be due to the supplementary effect of the dams' milk. Shaw *et al.* (Shaw, Enfield, and Wollman, *J. Nutrition*, **67**:253, 1959) reported a reduction in the amount of caries in groups of animals receiving a supplement of dairy products to an otherwise cariogenic diet. If this decrease in caries is indeed due to the supplementation of this cariogenic diet by the dams' milk, it might be suspected that the cariogenic diet used in this experiment was not adequate for the rat at this stage of its development. Further studies are required to determine whether other factors, such as bacterial or emotional, are contributing to the observed difference between groups.

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This investigation was supported by Research Grant D-254 (C2) from the N.I.D.R., U.S. Public Health Service.

Pathogenicity Experiments with the Flora of the Periodontium in Rice Rats

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Periodontal disease in the rice rat has been shown to be histologically comparable to periodontal disease in man (Gupta and Shaw, *Oral Surg.*, **9**:592, 1956). However, it has been demonstrated that the resident flora of the periodontium in the rice rat differs strikingly from that of man (Macdonald, Socransky, and Sawyer, *Arch. Oral Biol.*, **1**:1, 1959). Since the gingival flora of periodontal disease in man is capable of producing characteristic mixed anaerobic infections in the subcutaneous tissues of the guinea pig (Rosebury and Foley, *J.A.D.A.*, **26**:1791, 1939), it appeared worthwhile to determine whether or not the periodontal flora in the rice rat would produce similar infections.

The molar teeth and associated periodontium from 11 rice rats, all with periodontal disease, were removed, and in each instance diluted 100 fold in heart infusion broth and ground in a glass tissue grinder. The 11 suspensions were injected subcutaneously into the groin of 350-gm. guinea pigs. Each suspension was injected into two animals in doses of 1.0 and 1.5 ml. In addition, 6 of the suspensions were cultured anaerobically as unstreaked drops on blood agar plates for 5 days. The growth from 2 drops was suspended in 3-ml. heart infusion broth and injected in 1-ml. amounts into 2 guinea pigs.

None of the total of 34 guinea pigs developed typical mixed infection of the type seen when gingival scrapings from man are used as inoculum (Rosebury and Foley, *J.A.D.A.*, **26**:1791, 1939). Seventeen guinea pigs developed no lesions in 7 days; 5 showed small abscesses (less than 1 cm. in diameter) filled with caseous exudate. All 12 guinea pigs injected with uncultured or cultured suspensions from 3 of the rice rats developed spreading acute cellulitis with little exudate. *Escherichia coli* was recovered in almost pure culture from these lesions. Pure culture of the organism in each instance was found to be capable of eliciting similar inflammatory lesions when injected into guinea pigs. These results provide evidence of additional fundamental differences between the flora of periodontal disease in man and in the rice rat.

Quantitative studies of the rice-rat periodontal flora have demonstrated that bacterial counts increase with increases in the severity of periodontal disease; the higher counts are largely attributable to increased numbers of enterococci (Socransky, Macdonald, Sawyer, and Auskaps, *Arch. Oral Biol.*, in press). The findings suggested the possibility of special significance of enterococci in the pathogenesis of periodontal disease in the rice rat. It therefore appeared desirable to determine the pathogenicity of these organisms in the subcutaneous tissues of this animal. Five strains of enterococci isolated from the oral cavity of rice rats were grown in trypticase soy broth. The cells were suspended in saline to give concentrations of 1, 5, and 10×10^9 cells/ml. Each strain was injected subcutaneously into the groin of young adult rice rats. One rice rat was inoculated with 1 ml. of bacterial suspension at each dosage level. Three of the strains, in addition, were heat-killed (70° C. for 60 min.) and inoculated into rice rats in the same dosage as above. All the animals were examined daily and sacrificed at 4 days.

No lesions developed in the control group injected with heat-killed enterococci. All but one of the 15 rice rats injected with viable cultures developed local abscesses containing a small amount of very thick exudate from which enterococci were cultured. One animal died during the first 24 hours and had inflammation and adhesions at the site of infection. These findings indicate that the enterococci are capable of infecting the subcutaneous tissues of the rice rat and lend further support to the view that these organisms may be significant in the pathogenesis of periodontal disease in the rice rat.

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The Use of Pentamethylentetrazol To Decrease Mortality during Collection of Saliva from Rats

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A method for the collection of large quantities of rat saliva has previously been reported (Bernarde, Fabian, Rosen, Hoppert, and Hunt, *J. D. Res.*, **35**:326, 1956). In this technic, salivary flow is induced by pilocarpine after anesthesia with pentobarbital sodium.* In recent experiments this method for collecting saliva resulted in 40 per cent mortality in Hunt-Hoppert caries-resistant rats and 10 per cent mortality in the Osborne-Mendel–susceptible (OMS) rats. The causes of death were believed to be an accumulation of fluid in the upper respiratory tract and depression of the medulla oblongata (especially the respiratory center) by pentobarbital.

Atropine sulfate was used to antagonize the action of pilocarpine, in order to stop the secretion of fluids into the upper respiratory tract. The use of atropine did not reduce mortality when given at the termination of saliva collection. Saliva was not produced when atropine was given before pilocarpine.

Promazine hydrochloride,† a tranquilizer, was next tried as a preanesthetic, in order to reduce the dosage of pentobarbital. Various ratios of this preanesthetic and pentobarbital were used. Results with this method indicated that, although mortality was slightly lessened, the amount of saliva collected was reduced. Pentamethylentetrazol,‡ a stimulant of the central nervous and respiratory systems, was then employed to counteract the depressant effect of pentobarbital upon the respiratory system.

A total of 60 Hunt-Hoppert caries-resistant rats of various ages was divided into two groups with the same number of littermates in each group. The first group was given an intraperitoneal injection of 30 mg/kg pentobarbital sodium. When they had become anesthesized, they were given 7.5 mg/kg pilocarpine hydrochloride subcutaneously and placed in the collection holder. The flow of saliva was directed into small graduated centrifuge tubes. Ten minutes after salivation began, a subcutaneous injection of 50 mg/kg pentamethylentetrazol was administered. The animals were removed from the collection holder 20 minutes after they started salivating. The second group of rats was given the same treatment, but pentamethylentetrazol was not administered. The animals were observed 24 hours later for survivors.

The animals receiving pentamethylentetrazol immediately tended to exhibit a faster rate of respiration, and some moved their heads briefly. Only 10 per cent (3 rats) of the pentamethylentetrazol-treated animals died, whereas 50 per cent (15 rats) of the controls died ($P < 0.01$). Two of the three experimental rats which did not survive died within seconds after receiving this drug. These two animals appeared very weak and might have responded if pentamethylentetrazol had been given earlier. The use of pentamethylentetrazol slightly reduced the amount of saliva collected, but the yield was sufficient for experimental use (see table).

Drug Treatment	No. of Rats	Average Saliva Produced per Rat (Ml.)	Per Cent Mortality
Pentobarbital, pilocarpine, and pentamethylentetrazol	30	2.61	10
Pentobarbital and pilocarpine	30	2.72	50

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* Nembutal, Abbott Laboratories.

† Sparine, Wyeth Laboratories.

‡ Metrazol, Knoll Pharmaceutical Company.

*Incidence, Ecology, and Reproduction of Oral Protozoa**

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Examination of 700 patients revealed 39 per cent harboring *Entamoeba gingivalis;* 23 per cent, *Trichomonas tenax;* and 17.7 per cent, both. Seventy-nine samples diagnosed as negative by original wet-smear examination were found positive on culturing (28, *E. gingivalis;* 51, *T. tenax*) in Balamuth's egg-yolk fluid medium (*Am. J. Clin. Pathol.,* **16**:380, 1946) overlaying Dobell and Laidlaw's solid egg slants (*Parasitology,* **18**:283, 1926) and in St. John's fluid medium (*Am. J. Trop. Med.,* **12**:301, 1932). Maximum growth of Protozoa was obtained at a pH of 7.6–7.8.

Findings in relation to diagnosis of mouth conditions were (1) relatively "clean and healthy" mouths (514 patients): *E. gingivalis,* 26.4 per cent; *T. tenax,* 11.2 per cent; both, 6.4 per cent; (2) early progressive stages of periodontosis (141 patients): *E. gingivalis,* 65.2 per cent; *T. tenax,* 48.2 per cent; both, 39 per cent; (3) advanced periodontosis (45 patients): *E. gingivalis,* 100 per cent; *T. tenax,* 80 per cent; both, 80 per cent.

Incidence of *E. gingivalis* and *T. tenax,* respectively, by age groups were (1) 23 children, six to twelve years: no Protozoa; (2) 145 teen-agers, thirteen to nineteen years: 16.5 and 6.2 per cent; (3) 175 patients, twenty to thirty years: 40.6 and 19.4 per cent; (4) 122 patients, thirty-one to forty years: 55.5 and 32.8 per cent; (5) 145 patients, forty-one to fifty years: 48.3 and 37.2 per cent; (6) 68 patients, fifty-one to sixty years: 47 and 29.4 per cent; (7) 22 patients, sixty-one to eighty years: 50 and 22.7 per cent.

In cultures in which both protozoans and rather large numbers of bacteria were present there occurred a definite alternation of ecological ascendancy. *Entamoeba gingivalis,* by phagocytic action, consumes both *T. tenax* and bacteria. *Trichomonas tenax* ingests bacteria and will also kill a few amoebae by a large number attacking an amoeba, resulting in disintegration of the latter. Both bacteria and flagellates reproduce at a faster rate than do amoebae. For a time *E. gingivalis* flourishes; then, with diminution of food supply, the population declines with only cysts persisting. *Trichomonas tenax* now assumes ascendancy until further reduction of bacteria by the trichomonads diminishes this food supply. The strain of flagellates becomes weakened, the amoebae excysts, dividing into numerous trophozoites, and proceed to feed upon the debilitated trichomonads, once again assuming population ascendancy.

Microscopic observation of living specimens and cytochemical reactions have repeatedly confirmed the existence of cysts of both *E. gingivalis* and *T. tenax,* as well as somatella of the latter. Cystic stages are commonly found only in mouth biomes and cultures with low floral populations. From such mouths and cultures (sterile fluid media replacement in culture vials every 48 hours), cysts of *E. gingivalis* show (1) nuclear mitotic divisions, forming from 2 to 8 nuclei; (2) gradual consumption of chromatoid bodies and glycogen masses; and, finally, (3) excystation of multinucleated trophozoites, with subsequent binary fissions resulting in numerous small (3–8 μ) amoebulae. Cysts of *T. tenax* also form from 2 to 8 nuclei, with subsequent or sometimes concomitant cytoplasmic fragmentation, giving rise to spherical somatella comprised of 2–8 young trophozoites which ultimately burst from the periplast envelope surrounding them and proceed to grow and reproduce by longitudinal binary fission. Refrigeration at 2°–6° C. promotes encystment of *T. tenax.* When numbers of bacteria are allowed to increase in cultures, early cystic forms of both protozoans revert to the trophozoite form, and both tend to assume the cystic form in a habitat largely devoid of bacteria.

Nuclear constituents and cytosomal elements such as chromatoid bodies and metachromatic granules were best demonstrated by Faust's rapid iron-hematoxylin method (E. C. Faust and P. F. Russell, *Clinical Parasitology* [6th ed.; Philadelphia: Lea & Febiger, 1957], p. 947). Glycogen masses were demonstrated by Donaldson's iodine-eosin reaction (*Lancet,* **1**:571, 1917).

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*Relation of Thermolabile Components to Optical Properties of Dental Enamel**

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In 1862 Hoppe (*Virchows Arch.*, **24**:13, 1862) observed that thin sections of human enamel underwent a change in sign of birefringence when heated to 800° C. These changes were later studied more extensively by Schmidt and Keil (W. J. Schmidt and A. Keil, *Die gesunden und die erkrankten Zahngewebe des Menschen und der Wirbeltiere im Polarisationsmikroskop* [Munich: Carl Hanser Verlag, 1958]), and by Cape and Kitchin (*J.A.D.A.*, **17**:193, 1930). Schmidt and Keil assumed that this change in sign was brought about by loss of water from submicroscopic pores, whereas Cape and Kitchin implied a relation to the loss of CO_2 by heating. Studies have been done in an attempt to correlate optical changes with loss of the heat-labile components of enamel, i.e., water, protein, and carbon dioxide.

Sections of enamel from upper anterior teeth from patients aged twenty to forty years were heated in an oven from room temperature to 980° C. by 100° intervals. Retardation was determined by using a polarizing microscope equipped with a Berek compensator. Measurements were made at external, central, and inner zones at each temperature. A total of six sections was studied. In three of the sections the average thickness was 35 ± 3 μ. In these last three sections, three readings were made at each zone, and a mean value was determined.

The weight loss of three portions (about 150 mg.) of 60-mesh enamel prepared by the bromoform method was determined over the same temperature intervals as those used for the optical studies. The samples were weighed to the nearest 0.01 mg. on a semimicrobalance.

Similar portions of the 60-mesh enamel were placed in a pyro-ceram combusion tube and heated slowly up to 1,000° C. The sample was washed continually with dried CO_2-free argon gas, which passed into a calcium hydroxide solution, where precipitation disclosed the temperature range over which carbon dioxide was evolved.

Between room temperature and 100° C. there was an increase in positive birefringence in all zones. From 100° to 200° the trend was momentarily reversed. Between 200° and 400° the birefringence became positive and at 400° assumed a maximum value of +0.007 as determined from section thickness. As heating was continued up to 1,000°, the positive birefringence diminished to nearly zero. In two sections there occurred a "reluctant" surface layer about 100 μ deep which remained negative long after the inner areas had assumed maximum positive values. All observations were made on the buccal surfaces; however, similar changes were observed in other areas of the enamel.

Weight-loss studies showed 1 per cent water loss between room temperature and 100° for moist enamel. Using the weight at 100° as a base, it was determined that the samples studied lost 0.83 per cent of their weight between 300° and 400° C. Careful heating studies of the enamel, using the Feigl spot test analysis (F. Feigl, *Spot Tests in Organic Analysis* [New York: Elsevier Co., 1956], p. 402) indicated that all the protein is burned off at 300° within 15 minutes. Inasmuch as the carbon dioxide studies show that CO_2 is evolved between 400° and 900°, it is assumed that this weight loss between 300° and 400° is due to the evolution of a form of "bound" water. Between 400° and 900° the samples lost 2.53 per cent of their weight. This loss is presumed to be mainly carbon dioxide, and the figure approximates those given by Little and Brudevold (*J. D. Res.*, **37**: 991, 1958).

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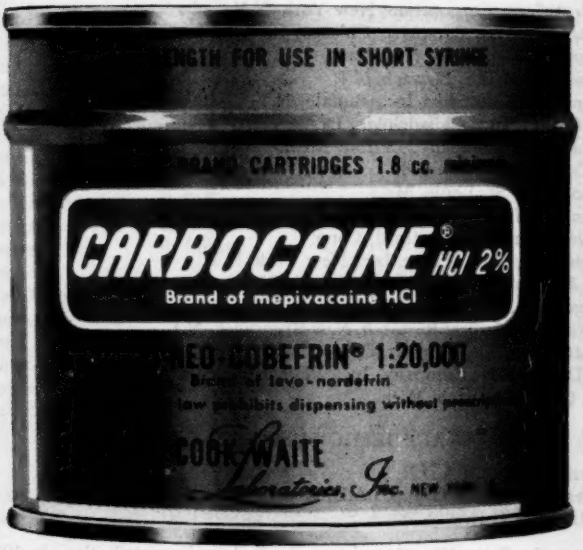

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